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February 13, 1998

BY HAND

Document Control Office (7407)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Room G-099
401 M Street, S.W.
Washington, D.C. 20460

Re: ECA Proposal for Methyl Methacrylate to Provide Alternate Testing to Meet HAPs Rule Testing Requirements, OPPTS-42187B; FRL-5742-2

Dear Sirs:

At the request of Mr. Leukroth, I am submitting for the HAPs ECA docket three copies of the Methacrylate Producers Association's (MPA's) testing proposal for methyl methacrylate. The proposal and the accompanying comments on EPA's proposed Section 4 testing of methyl methacrylate were previously filed in docket OPPTS 42187A, FRL 4869-1 on December 10, 1997.

Yours truly,

L. Mark Wine
Counsel for MPA

cc (via mail w/o encs):

Richard W. Leukroth, Jr.
Chemical Control Division (7405)

Container No CBI

METHACRYLATE PRODUCERS ASSOCIATION, INC.
1350 Eye Street, N.W., Washington, D.C. 20005
Office: (202) 962-9400 Facsimile: (202) 289-3565

December 10, 1997

BY HAND

Charles M. Auer, Director
Chemical Control Division
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Room E513C, Mail Code 7405
Washington, D.C. 20460

Re: Proposed Test Rule for Methyl Methacrylate
OPPTS-42187A; FRL-4869-1

Dear Mr. Auer:

The Methacrylate Producers Association, Inc. (MPA) urges EPA not to issue its proposed test rule for methyl methacrylate (MMA). As detailed in our attached comments, the substantially completed Toxicological Review of MMA, developed as part of the Agency's IRIS Pilot Program, finds the existing MMA database fully adequate to assess inhalation risks and to establish an up-to-date Reference Concentration (RfC).

MPA is, nonetheless, willing to conduct an oral neurotoxicity study with special attention to reproductive target organs to supplement the existing database. Although these data are not necessary for risk assessment, MPA agrees they would provide useful information. We would thus be willing to enter an Enforceable Consent Agreement (ECA) to conduct such testing. Because the testing we propose would incorporate elements not included in standard EPA protocols, agreement to conduct such testing would best be achieved through an ECA.

EPA's IRIS Draft Review derives an RfC for MMA in which the Agency has "medium to high confidence." The RfC is based on a "high" quality long-term rat inhalation study for which additional histopathologic examinations were commissioned by MPA. EPA finds the overall database (including developmental studies it finds "acceptable" in both mice and rats) strong enough that "[n]o uncertainty factor is applied to the RfC for database deficiencies." The Agency further finds that the uncertainty factor it employs to account for interspecies extrapolation may be able to be reduced once it is able to review the PBPK model developed for MPA by Dr. Melvin Andersen at EPA's request.

The comprehensive assessment of the MMA database in EPA's recent IRIS Review -- in contrast to the cursory review in the HAPs proposal support document -- establishes the absence of necessity for conduct of further studies to assess MMA risks. Without a need for further studies, a TSCA Section 4 rule cannot be justified.

MPA urges EPA to work with it to develop an ECA for conduct of a neurotoxicity study on MMA, rather than unnecessary preparation of an unwarranted Section 4 rule.

Sincerely,

Elizabeth K. Hunt
Executive Director

Attachment

cc: TSCA Docket (three copies with Exhibits attached) (by hand)
Dr. Annie Jarabek (w/o Exhibits) (by mail)
Richard W. Leukroth, Jr. (w/o Exhibits) (by mail)
Dr. Jeffrey Gift (w/o Exhibits) (by mail)

UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY

PROPOSED TEST RULE FOR HAZARDOUS AIR POLLUTANTS	)	OPPTS–42187A; FRL–4869-1
61 Fed. Reg. 33178 (June 26, 1996)	)	

COMMENTS AND PROPOSAL FOR AN ENFORCEABLE CONSENT AGREEMENT OF THE METHACRYLATE PRODUCERS ASSOCIATION, INC.

Elizabeth K. Hunt
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December 10, 1997

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I. INTRODUCTION AND SUMMARY.

The Methacrylate Producers Association, Inc. (MPA) -- whose member companies are CYRO Industries Inc., Elf Atochem N.A., Inc., ICI Acrylics, Inc., and the Rohm and Haas Company -- has been a leader in sponsoring testing for methyl methacrylate (MMA) and other methacrylates. MPA, its member companies, and its European industry counterparts have spent millions of dollars on these voluntary efforts. MPA urges EPA to recognize that it cannot justify the extensive multi-million dollar TSCA Section 4 testing program it proposes for MMA.[1]

While the existing data are fully adequate for assessing MMA residual risks, MPA is willing to conduct an appropriate neurotoxicity study and is willing to incorporate assessment of appropriate reproductive target organs in that test. We urge EPA to accept MPA's alternate testing approach as detailed in our comments and to work with us to develop an Enforceable Consent Agreement (ECA) for conduct of such a study, rather than unnecessary preparation of an unwarranted and potentially contentious Section 4 rule.

The data on MMA are so extensive that it takes 167 pages to describe the findings in the European Centre for Ecotoxicology and Toxicology of Chemicals Joint Assessment of Commodity Chemicals (JACC) Document on MMA (February 1995) (attached as Exhibit A). The bibliography alone comprises over thirty pages of small type, reflecting more than 750 references. The JACC Document describes (in Table 18, pp. 53-55) the 46 repeat dose *inhalation* studies in rats, mice, guinea pigs and dogs, including the National Toxicology Program's 1986 lifetime bioassays in mice and rats. An Executive Summary of these data prepared by the MPA Science Committee

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[1] MPA shares the concerns regarding generic legal and scientific deficiencies in the overall HAPs proposal as set forth in comments filed by the Chemical Manufacturers Association (CMA). We endorse those comments that are relevant to MMA, including especially several of the comments on testing protocols. CMA previously identified many serious technical issues and deficiencies in these protocols. See CMA Comments (Sept. 19, 1996).

is attached as Exhibit B. This extensive database is contrary to EPA's conclusion that substantial additional testing is necessary to assess MMA inhalation risks.

The nearly completed IRIS review of MMA shows that EPA can and has thoroughly assessed MMA inhalation risk potential with the existing database. As EPA's IRIS Draft[2] (Exhibit C at p. 75) notes: "[N]o uncertainty factor is applied to the RfC for database deficiencies." If there is any doubt of the Agency's risk assessment needs, MPA urges EPA to seek the assistance of the Science Advisory Board before completing this rulemaking.

As the IRIS Draft recognizes, MMA has been shown not to pose any carcinogenic, developmental, or serious acute inhalation hazards at ambient or workplace exposure levels. It is thus not surprising that EPA ranked MMA near the bottom of its ranking of all Clean Air Act hazardous air pollutants in its proposed Section 112(g) rule. See 59 Fed. Reg. 15504 (April 1, 1994). EPA's own analyses confirm that MMA is not a material that poses the type of special risk that might justify extensive additional testing.

The existing MMA database addresses virtually all of the proposed testing end-points:

- *Acute Inhalation Testing*: Numerous acute inhalation studies have been conducted with consistent results. Further studies will be of no value for ppm-level Clean Air Act residual risk analyses.

- *Respiratory Sensory Irritation*: A recent, well-executed respiratory sensory irritation (RD_{50}) study (not referenced by EPA) demonstrates that MMA is not a respiratory sensory irritant.

- *Developmental Toxicity*: At least seven developmental toxicity studies have been performed via the inhalation route in both rats and mice. These studies have shown that MMA is not a potential teratogen.

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[2] EPA Draft IRIS Support Document, "Toxicological Review: Methyl Methacrylate" (Feb. 10, 1997).

- *Reproductive Toxicity*: Existing data indicate that reproductive toxicity is not an endpoint of concern for MMA. Under criteria used by OECD in its Screening Information Data Set (SIDS) program, there are sufficient test data to assess reproductive toxicity. EPA's recent draft IRIS document agrees that the absence of a reproduction study is not significant to risk assessment. Any remaining questions will be addressed by special attention to reproductive target organs in the neurotoxicity study MPA is willing to conduct.

- *Immunotoxicity*: MMA is a recognized human dermal sensitizer. Further immunological characterization of MMA is unnecessary.

- *Neurotoxicity*: MPA is willing to conduct an additional neurotoxicity study by the oral route to supplement the existing database.

II. THE EXISTING DATABASE PROVIDES ADEQUATE INFORMATION TO ASSESS MMA'S POTENTIAL RISK.

After reviewing the existing database, EPA selected MMA for inclusion in its IRIS Pilot Program (61 Fed. Reg. 14570, April 2, 1996). That extensive EPA risk assessment program has now completed a Draft Toxicological Review that finds sufficient data to have "medium to high confidence" in the proposed Reference Concentration (RfC) (Exhibit C). The IRIS review shows that the available data are sufficient for EPA's risk assessment needs. In addition, MPA has prepared a PBPK risk assessment model not yet reviewed by EPA that can be used without the need for more costly descriptive toxicology. Moreover, when EPA undertook its risk-based review of all hazardous air pollutants, it ranked MMA near the bottom of the list based on an analysis that found it had low toxicity (59 Fed. Reg. 15504, 15549-1563 April 1, 1994) (summary of method for evaluating relative toxicity of hazardous air pollutants).

These existing assessments are consistent with MPA's Science Committee's summary of the MMA database (Exhibit B):

> Methyl methacrylate (MMA) is a weak sensitizer and an irritant to the skin, eyes, and the respiratory tract. Experience in man suggests that there is no clear evidence that MMA is hazardous during manufacture or use in a number of industries. No genotoxic, carcinogenic, reproductive or teratogenic effects have been reported in exposed populations. The pungent odor and the irritant nature of MMA serve as a warning property and tend to keep inhalation exposures low. These observations agree with the lack of genotoxic, carcinogenic, reproductive or teratogenic effects observed in laboratory studies on MMA. Based on the large database, the likelihood of any other short- or long-term health effects is limited.

A. EPA Has Recently Found the Substantial MMA Toxicity Database Adequate to Assess Inhalation Risks.

The MMA database is robust and fully adequate to assess risks. EPA selected MMA as one of only 13 compounds for its IRIS Pilot Program (61 Fed. Reg. 14570, April 2, 1996). A year later it completed a Draft Toxicological Review (February 10, 1997) that (at p. 74) placed "medium to high confidence" in its proposed RfC.

As EPA's IRIS Draft points out (at pp. 74-75), the proposed RfC was based on the high quality Hazleton Laboratories long-term rat inhalation study (whose original findings were supplemented by more recent histopathologic analyses sponsored by MPA). The Draft also finds (at p. 75) that no uncertainty factor needs to be applied to the RfC to account for database deficiencies -- as none were identified.

In short, EPA has already determined an RfC based on a database it considers sufficiently complete. The database needed for regulatory assessments already exists. The IRIS process of identifying an RfC is remarkably similar to the Agency's proposed plans for residual risk evaluation under the Clean Air Act and indeed is aimed specifically at providing the needed toxicity guidance for such regulatory assessments:

> Combined with specific situational exposure assessment information, the summary health hazard information in IRIS may be used as a source in evaluating potential public health risk from environmental contaminants. (*Id.*)

If there is any doubt about the Agency's risk assessment data needs, MPA urges EPA to seek the advice of its Science Advisory Board (SAB) before the current rulemaking is concluded. This HAPs test rule and the anticipated subsequent test rule proposals fall within the ambit of the SAB's charter "to provide independent advice to EPA's Administrator on the scientific and technical aspects of environmental problems and issues" (EPA Advisory Committee Charter, Science Advisory Board, Sec. 3). Such consultation and advice would be particularly appropriate here given the SAB's special expertise and experience in risk assessment and Clean Air Act evaluation.

In the meantime, by moving forward with its IRIS evaluation, the Agency has shown that the MMA data are fully sufficient to complete an inhalation risk evaluation.[3] The extensive additional testing in the Proposed Rule is not justified.

B. The PBPK Model Previously Submitted to EPA by MPA Provides a Strong, Scientifically Sound Basis for Further Improvements in MMA Risk Assessment.

At the suggestion of EPA scientists, MPA retained Dr. Melvin Andersen of ICF-Kaiser to work with Dr. Clay Frederick of MPA-member Rohm and Haas to develop a physiologically-based pharmacokinetic (PBPK) model for MMA dosimetry in nasal tissues. That model (Exhibit D) was submitted to EPA for use in the IRIS Pilot Project on September 26, 1996.

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[3] It bears noting that EPA has yet to issue its § 112(f) report indicating how it will determine, in residual risk determinations, whether there is an "ample margin of safety" for non-carcinogens -- so it is impossible to know yet exactly what data the Agency will believe is necessary. Given the fact, however, as we detail in these comments, that the existing and proposed data are fully adequate for EPA to derive an RfC, that database is certainly more than adequate for the contemplated regulatory assessments.

MPA was informed by Dr. William Farland of EPA that, for administrative and timing reasons unique to the IRIS Pilot Project, the model could not be reviewed or evaluated by EPA scientists for use in the IRIS documentation. The Draft IRIS Review, nonetheless, notes (at p. 76) that these PBPK data will be valuable in achieving reductions in future risk assessments in inter-species dosimetric and toxicokinetic uncertainty.

EPA is familiar with the model's methodology. The model draws significantly on the models for vinyl acetate and acrylic acid, both of which have been reviewed in detail by EPA in scientific workshops organized over the last several years. Another such joint workshop that will specifically address the MMA model will be held in February.

III. THE AGENCY HAS NOT MET THE LEGAL CRITERIA OF TSCA SECTION 4.

TSCA Section 4 testing can be required only when EPA has provided substantial evidence (TSCA Section 19(c)(1)(B)(i)) of potential significant risk (an "A" finding) or substantial exposure (a "B" finding) *and*, in either case, a documented need for additional data to assess unreasonable risk. For MMA, EPA's overall evidentiary showing is legally insufficient.[4] The proposed testing program has not been and cannot be justified to meet any governmental risk assessment needs, especially since EPA itself is nearing completion of a comprehensive MMA review in the IRIS Pilot Project.

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[4] We do not contest EPA's assertions that MMA production volume, and the estimate of exposed workers and of releases, exceed the Agency's previously established definitions of significant exposure or release (*i.e.*, production of more than one billion pounds, more than 100,000 exposed workers, and more than two million pounds released). What we do question is the asserted need for more toxicity testing to assess risks posed by any of this exposure.

To make an "A" finding (TSCA Section 4(a)(1)(A)(I)), EPA needs to show MMA may present an unreasonable risk, *i.e.*, "an existing possibility of harm [that] raises reasonable and legitimate cause for concern," Ausimont U.S.A. v. EPA, 838 F. 2d 93, 96-97 (3d Cir. 1988). The Agency must "accumulat[e] enough information to demonstrate a more than theoretical basis for suspecting ... some amount of exposure takes place and that the substance is sufficiently toxic at that level of exposure to present an 'unreasonable risk of injury to health.'" CMA v. EPA, 859 F.2d 977, 984-85, 986 (D.C. Cir. 1988). As EPA said in its aryl phosphate proposed test rule, testing should be required *only* where "human or environmental exposure is of such magnitude or type that it may need to be regulated if test data reveal adverse effects." 57 Fed. Reg. 2138, 2144 (Jan. 17, 1992).

Even with an "A" or "B" finding, EPA must with substantial evidence demonstrate why the proposed testing is "necessary" for the "effects of" MMA "on health or the environment [to] reasonably be determined or predicted" (Sections 4(a)((1)(A)(ii) - (iii) and (B)(ii) - (iii)).

In determining what data are necessary, it is essential to do so in the context of the exposures to be assessed. MMA has a strong, pungent odor. As a result, MMA airborne concentrations are kept low to avoid worker and neighbor odor complaints. Workplace exposures are limited by both a TLV® of 100 ppm, and, perhaps more importantly, an odor threshold of 0.21 to 0.083 ppm -- more than two orders of magnitude below the TLV (JACC at p. 5).

Only 19 facilities reported 1995 TRI emissions greater than the ten-ton major source definition. These and the lesser emitters of MMA are very unlikely to be causing fenceline exposures of significance.

As we demonstrate below -- with respect to each EPA-proposed test -- the Agency has not met its obligation under Section 4 to explain why any of the data gaps it identifies are

important to its risk assessment needs. Nowhere does it demonstrate with substantial evidence that any of the proposed studies are necessary to assess Clean Air Act residual risks. EPA's proposed testing program is not justified under the legal criteria of TSCA Section 4. With the neurotoxicity testing and reproductive organ pathology that we are willing to conduct, the MMA database will be more than adequate to assess inhalation risks.

IV. ALTHOUGH THE EPA-PROPOSED TESTING OF MMA IS NOT JUSTIFIED, MPA IS WILLING TO CONDUCT, UNDER AN ENFORCEABLE CONSENT AGREEMENT, AN ORAL 90-DAY NEUROTOXICITY TEST WITH REPRODUCTIVE TARGET ORGAN PATHOLOGY TO SUPPLEMENT THE EXISTING DATABASE.

In proposing this test rule, EPA did not sufficiently review the extensive data available on MMA, nor provide sufficient scientific or legal reasons to support the proposed testing. The available data, the imminent pendency of a revised RfC for MMA developed under the IRIS Pilot Project, and the availability of a PBPK model each demonstrate that further testing is not necessary to assess MMA risks for Clean Air Act or other regulatory assessment purposes.

Nonetheless, MPA recognizes that the literature raises some questions concerning MMA's neurotoxic potential and is therefore prepared to supplement the existing database with a neurotoxicity test by the oral route. MPA is also willing to add to the neurotoxicity test selected reproductive target organ pathology to supplement the extensive developmental and reproductive organ data that are already available.

A. Neurotoxicity

Several lifetime MMA inhalation studies in rodents at high concentrations have shown irritation, but no overt effects on behavior and no frank neurological histopathology, at

exposure concentrations up to 500 ppm (female rats) or 1000 ppm (male rats, male and female mice) (NTP 1986). The literature suggests that MMA slows nerve conduction velocity in animals and reports paresthesia (numbness and tingling in fingertips) in humans following direct repeated skin contact with MMA. A full description of the MMA neurotoxicity database is in the attached JACC Report (Exhibit A at pp. 82-87).

Although MPA believes that the data show these neurotoxic effects to be direct, local, anesthetic-like, and reversible, it is willing to conduct an appropriate study to investigate potential systemic neurotoxicity. This study should be by the oral route. Existing data adequately characterize the toxicity of MMA to the respiratory tract and show point-of-contact effects. Any further risk assessment and regulation will certainly control exposures to protect these tissues. The neurotoxicity test dosing strategy should thus be based on the expected delivery of MMA and its hydrolysis product, methacrylic acid, to target tissues within the nervous system. Oral dosing will deliver more chemical to the systemic tissues of concern for neurotoxicity evaluation.

Because the inhalation NOAEL in rats for inhaled MMA is 25 ppm, or 10.58 mg/kg,[5] any neurotoxicity test by inhalation would need to be conducted at such minimally toxic concentrations (*i.e.*, between 25 and 50 ppm). Rats exposed by inhalation for two years to 100 ppm MMA developed overt degeneration of the olfactory epithelium. Exposure at this concentration is equivalent to 42.32 mg/kg. Indeed, the irritating effects of MMA inhalation doses above 100 ppm would likely be sufficiently adverse to the animals to confound interpretation of the functional observation battery and other behavioral observations. Neurotoxicity tests with MMA conducted

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[5] Rohm and Haas, 1979. The inhaled dose during a 6 hour exposure to 25 ppm in a 300 g rat, based on US EPA ventilation parameters, is: amount inhaled = 25 ppm*88/24.45/1000*0.0981/min*60 min/hr*6 hr/0.3 kg = 10.58 mg/kg.

by oral dosing strategies, on the other hand, could be conducted at a daily dose in this general range. Whether the dosing should be through feed, drinking water or gavage raises technical issues of palatability and local irritation that we would like to discuss with the Agency.

B. Reproductive Toxicity

EPA calls for reproductive testing solely on the basis that no multi-generation tests have been conducted; it points to no study suggesting adverse reproductive effects are likely.

Several 90-day and lifetime MMA studies in multiple species, which include histopathologic examination of tissues from the urogenital/reproductive systems, have been completed. And, as noted below, at least seven inhalation developmental studies have been conducted. In addition, a dominant lethal study at inhalation doses up to 9000 ppm for five days found no significant differences in fertility of the treated males or in survival, total implants and early or late post-implantation death in the offspring of treated males (ICI 1976).

Under the internationally-recognized SIDS screening criteria for reproductive toxicity, the existing MMA data provide an adequate basis to analyze potential reproductive effects. See OECD SIDS Manual, 2nd revision, May, 1996 at page 5 (attached as Exhibit E). ("When a 90-day repeated dose study is available and is sufficiently documented with respect to studying effects in the reproductive organs and a developmental study is available, the requirements for the reproduction toxicity endpoint would be satisfied.")

In the absence of any indication that MMA poses a potential reproductive toxicity risk, and in light of MMA's general lack of toxicity, and because it does not bioconcentrate, MMA risk evaluation can be completed without a multi-generation reproductive study. This "data gap" is of no significance to RfC risk assessment. EPA must focus on true data "needs," not box-

checking gaps. As EPA's IRIS Review (at p. 75) concludes: "MMA is so reactive at the portal of entry [that] the potential for systemic effects is deemed remote."

Despite the absence of any risk assessment need for more reproductive findings, we are willing to include with the neurotoxicity study special attention to appropriate reproductive target organs. We would like to discuss the appropriate tissues with the Agency as part of protocol development for the neurotoxicity study.

C. Developmental Toxicity

EPA cites no studies that raise concerns about developmental effects of MMA. It asserts, though, that the database is inadequate because the three concededly adequate rat studies are not also accompanied by an adequate mouse study (Rohm and Haas 1976 is deemed inadequate because MMA was administered only through day 13) or a non-rodent species (the rabbit). This analysis gives insufficient attention to the weight of the existing data and overlooks an i.p. study in rabbits.

Seven MMA inhalation developmental studies have been performed in rats and mice. In contrast to the conclusions in the HAPs review -- EPA's IRIS Draft (at p. 75) finds that "[a]cceptable developmental studies were carried out in two species, rats and mice." Moreover, an i.p. study in rabbits that found no teratogenic effects at doses up to 0.4 ml/kg/d on days 6 to 18 of pregnancy was not evaluated by EPA (ICI 1976, discussed in JACC at 81-82). While i.p. injection is not an appropriate route of administration for teratology, the data from this study are further support for the conclusion that more developmental data are not needed.

The most definitive study, conducted in accordance with EPA and OECD guidelines, under GLP, is Solomon *et al* (1993). No teratogenicity, embryotoxicity or fetotoxicity was observed

in rats at exposure levels in excess of 2,000 ppm. The ECETOC review (JACC at 3) concluded that MMA "is not teratogenic in rats and mice exposed by the inhalation route."

When extensive developmental toxicity studies have been completed on rats, mice and other species at high doses -- with negative results -- and when there is no other evidence suggesting that MMA poses risk as a developmental toxin, further testing cannot be justified.

D. Acute Toxicity and Respiratory Sensory Irritation

EPA's "A" finding mentions pulmonary damage after 2-, 3- and 4-hour rat exposures at 97 ppm (Raje 1985) and slight irritation of the respiratory tract at 13.4 mg/ml and higher doses (Haskell Laboratory 1989); its finding of inadequate data to assess acute risks cites limitations in two studies that found respiratory irritation: the Raje study (too few animals, males only, single exposure level) and Oberly and Tansy 1985 (males only, only two exposure levels, limited endpoints reviewed). The Agency states it could find no data on respiratory sensory irritation.

The recent, comprehensive MMA respiratory sensory irritation (RD_{50}) study, at doses up to the limit of MMA explosivity, should satisfy EPA's needs. Stadler (1993) (attached as Exhibit F) found MMA is not a respiratory sensory irritant. This test makes unnecessary additional respiratory sensory irritation testing.

As attached Tables A and B demonstrate, MMA's acute inhalation toxicity has been extensively studied in the rat, mouse and other species for periods of one to eight hours. These tests show generally consistent results across several species that argue strongly for their validity.

MPA also objects to the acute inhalation study protocol and incorporates the CMA comments. The procedure described in OPPTS § 870.1350 (to be codified at 40 C.F.R. § 799.9135) is extremely expensive and wasteful of laboratory and animal resources. The BALF lavage

approach is a useful research tool, but it is not appropriate under a Section 4 test rule. If the objective of this guideline is to assess acute respiratory tract toxicity, classical toxicological approaches are still appropriate for identifying target organs and assessing reversibility of any histopathologic lesions, including those in the respiratory tract.[6]

Finally, MPA questions the need for additional *acute* inhalation testing when the Agency's stated use for its results is to undertake a residual risk analysis that will focus on the *chronic* effects of routine and continuous emissions. Clean Air Act Section 112(b) emphasizes this reality by not allowing EPA to consider accidental releases in Hazardous Air Pollutant listing and delisting decisions. Nor would acute testing be relevant to the Agency's accident release prevention program under Clean Air Act Section 112(r), as MMA is not one of the regulated acutely hazardous substances.

E. Immunotoxicity

EPA seeks immunotoxicity testing solely on the basis that no existing studies assess immune function.

Human clinical studies and animal testing suggest that MMA stimulates, not suppresses, the immune system (JACC at pp. 43-51). These studies support a t-Cell mediated immune response resulting in delayed contact hypersensitivity. MMA is presumed also to stimulate humoral immunity through IgG production like other skin sensitizers. Nonetheless, under both

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[6] MPA also endorses the other protocol-related concerns that CMA has described in its comments, including the requirement for determining alveolar macrophage phagocytic activity, the inappropriate reliance on simple exposure studies for screening purposes, the possibility of sampling errors, and the questionable use of such data for risk assessment purposes.

clinical and experimental conditions, the immune system is completely functional. Several 90-day and chronic studies with histopathology show no deleterious effect on the thymus, spleen or lymph nodes in rats, mice, and dogs (JACC at pp. 52-59).

In light of these data, no further testing should be required, especially given the lack of validation for EPA's proposed immunotoxicity testing and the lack of a defined process for using immunotoxicity testing results in a risk assessment. EPA's testing guidelines are established to detect immune deficiencies. As such, it is not reasonable to assay MMA further for immunotoxicity. In addition, interpretation of studies that demonstrate immunosuppression without also affecting histopathology or lymph node/spleen organ weights would be complex.

F. Oral Testing Is the Preferred Methodology for the Proposed Neurotoxicity Testing

EPA's original proposal provided an opportunity to use pharmacokinetics modeling to extrapolate *existing* oral data to inhalation scenarios, thereby avoiding the need to undertake inhalation testing (61 Fed. Reg. at 33188). EPA's recent favorable responses to several proposals to fulfill test rule requests through PBPK work and new oral studies recognizes the value of using PBPK data in *future* toxicological study design. As noted above, the neurotoxicity testing we propose would be more meaningful to risk assessment needs if it were conducted by the oral route. The oral route avoids extensive respiratory tract toxicity in nasal tissues, while providing higher exposure to systemic tissues throughout the body. It would certainly be arbitrary for EPA to allow use of PBPK modeling of *existing* oral data to assess inhalation risks, while not allowing *prospective* oral studies to fill assessment needs.

By closing the door to oral testing, EPA would obtain less valuable data at significantly increased testing costs. Oral dosing will result in higher delivered systemic doses than any reasonable inhalation dose that adequately takes into account the irritancy of MMA. Compared to other exposure routes, testing by inhalation involves considerably more manpower, analytical, and method development to establish exposure conditions, perform daily set-up and generate 6-hour/day exposures, and to maintain target concentrations with analytical verification. MPA estimates that requiring inhalation-based testing would more than double the cost of the proposed tests without any offsetting benefit.

V. CONCLUSION.

The toxicology database on MMA is robust and the tests proposed by EPA are not needed. As EPA's draft IRIS review has found, the existing database is sufficient to assess inhalation risks. Nonetheless, MPA is willing to conduct an oral neurotoxicity test with reproductive target organ pathology to supplement the extensive MMA database.

We urge EPA to accept MPA's alternate testing approach and to work with us to develop an Enforceable Consent Agreement, rather than unnecessary preparation of an unwarranted Section 4 rule. We believe our proposed study would provide useful information and are willing to initiate it expeditiously.

TABLE A

Acute Inhalation Toxicity (LC_{50} Values)

Species	Time	LC_{50} (ppm)	Reference
Rat	2	10,800-16,800[a]	Rohm and Haas, 1958
	4	7,093	Tansy, *et al*, 1980c: Oberly and Tansy, 1985
Rat	<8	3,760	Deichmann, 1941
	Unknown	10,910	Ouyang *et al*, 1988
	2	11,250-12,500	Borzelleca *et al*, 1964
Mouse	3	13,200	Spealman, 1945
	Unknown	7,416	Ouyang *et al*, 1988

a Reported as 45-70 mg/l

TABLE B

Acute Inhalation Toxicity Data

Species	Time (h)	Concentration (ppm)	Lethality (%) or parameter	Reference
Rat	1	9,800-17,800[a]	0	Kelly, 1993
	1	400	0	Innes, 1979
	4	4,632	0	NTP, 1986
	4	16,000	100 (_)	NTP, 1986
	4	16,000	80 (_)	NTP, 1986
	5	4,600	100	Deichmann, 1941
	8	1,200	0	Deichmann, 1941
	8	7,200-7,900[b]	70	Du Pont, 1937
Mouse	3	6,290[c]	5	Spealman, *et al*, 1945
	3	11,450[d]	13	Spealman, *et al*, 1945
	3	14,830[e]	100	Spealman, *et al*, 1945
	5	11,450[d]	60	Spealman, *et al*, 1945
	4	4,632	0	NTP, 1986
	4	16,000	100	NTP, 1986
	2	3,600-4,800	Min LC	Karpov, 1954 a,b
Mouse	N/A	27,650	56 min LT_{50}	Lawrence *et al*, 1974
Guinea Pig	4.25	17,300[f]	100	Spealman, *et al*, 1945
	5	4,600	100	Deichmann, 1941
	8	4,200	0	Deichmann, 1941
Rabbit	3.5	4,560	100	Deichmann, 1941
	4.5	4,200	100	Deichmann, 1941
	5	4,600	100	Deichmann, 1941
Cat	N/A	3,600-4,800	0	Karpov, 1954, a,b
Dog	1.5	17,300[f]	100	Spealman, *et al*, 1945
	3	9,890[g]	100	Spealman, *et al*, 1945

a-g Reported as: a, 41-74 mg/l; b, 30-33 mg/l; c, 26.2 mg/l; d, 47.7 mg/l; e, 61.8 mg/l; f, 72.1 mg/l; g, 41.2 mg/l

Exhibit A

European Centre for Ecotoxicology and Toxicology of Chemicals, Joint Assessment of Commodity Chemicals (JACC) Document on MMA (February 1995)

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Exhibit B

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METHYL METHACRYLATE

EXECUTIVE SUMMARY

Methyl methacrylate (MMA) is a clear, colorless, flammable liquid monomer with a pungent odor, widely used to produce methacrylic polymers, resins, and plastics such as those found in acrylic sheets, building materials, molding powders, and medical or dental devices. Extensive testing shows MMA does not pose an unreasonable hazard to human health or the environment.

Methyl methacrylate has low acute toxicity by the oral, dermal or inhalation routes. It is irritating to the skin, eyes, and mucous membranes of the respiratory tract. Methyl methacrylate is a skin sensitizer and may cross-react in individuals with prior exposure to other acrylic or methacrylic esters.

Metabolism of MMA is assumed to be similar in both man and experimental animals. Methyl methacrylate is rapidly absorbed and distributed in experimental animals following oral and inhalation exposure. *In vitro* absorption studies with human skin indicate MMA may be absorbed through the skin. However, even under occluded dermal conditions in animal studies, MMA is not absorbed in lethal quantities. After oral or parenteral administration, MMA is rapidly hydrolyzed to methacrylic acid (MAA) and methanol, with hydrolysis products further metabolized by normal physiological pathways (i.e., via methylmalonyl-coenzyme and the tricarboxylic acid pathways). In rats, about 80% of an administered dose of MMA is rapidly converted to CO_2 and eliminated. Conjugation with glutathione or tissue non-protein sulphydryl (NPSH) groups occurs to a minor extent when tissue concentrations of MMA are high and hydrolysis pathways reach saturation. Approximately 10 to 20% of inhaled MMA is deposited in the upper respiratory tract where it is metabolized by local tissue esterases and/or conjugated with tissue NPSH.

Subchronic exposures of rats and mice to MMA by oral and inhalation routes show local effects consistent with its irritant properties. For example, by inhalation, dose-related lesions are produced primarily in the upper respiratory tract (including rhinitis, inflammation associated with necrosis and loss of olfactory epithelium in the nasal turbinates), and lung congestion. Exposure to high atmospheric concentrations of MMA (> 1,000 ppm rats and mice, > 10,000 ppm dogs), produced body weight decreases and degenerative or necrotic changes in liver, kidney, brain, spleen, and bone marrow. Changes in the activities of liver enzymes were observed in animals exposed to relatively low concentrations of MMA. Inconsistent data have been reported on effects to the cardiovascular system, such as hypotension reported during artificial hip implantation surgery. Single studies suggest that inhalation exposure to high concentrations of MMA may induce behavioral or neurochemical changes, however, central nervous system effects have not been reported in chronic studies with exposure levels up to 1,000 ppm. Methyl methacrylate has been reported in humans to produce paresthesia, or numbness and tingling of exposed fingers, believed to be a local, reversible, anesthetic-like effect.

Methyl methacrylate shows no carcinogenic potential in experimental animals. Methyl methacrylate was not carcinogenic after lifetime inhalation exposures to concentrations up to 500 or 1000 ppm in rats or mice, respectively (NTP, 1986). Similarly, MMA was shown not to be carcinogenic in hamsters after lifetime inhalation exposures up to 400 ppm (Reno et al., 1979). In these studies, neither systemic effects nor neoplastic or pre-neoplastic changes were produced. Non-neoplastic changes restricted to the upper respiratory tract included rhinitis, serous and suppurative inflammation, epithelial hyperplasia, and degeneration of the olfactory epithelium. Since 10 to 20% of MMA has been shown to deposit in the upper respiratory tract, it is speculated that these changes result from hydrolytic cleavage of MMA, giving rise to MAA (a corrosive) and methanol. The primary involvement of the olfactory rather than the respiratory epithelium is consistent with the tissue distribution of esterases and air flow parameters in the rodent nasal turbinates. The respiratory epithelium is not affected. Due to the differences in the upper respiratory system anatomy and local tissue esterase activities between human and rodents, it is expected that human beings are less sensitive to the observed changes in the upper respiratory tract than rodents.

Chronic oral exposure to rats and dogs by capsule or in drinking water at concentrations up to 2000 ppm for 2 years produced no histopathological changes or treatment-related lesions (Borzelleca et al., 1964). Reported kidney weight increases are speculated to reflect an increase in specific acid transporter proteins associated with excretion on intermediate metabolites during clearance.

Methyl methacrylate is not mutagenic to Salmonella bacteria even at cytotoxic concentrations. *In vitro* systems which measure chromosomal aberrations such as the Mouse Lymphoma assay, suggest MMA has clastogenic activity, but this appears to be an indirect effect secondary to cytotoxicity. However, MMA is not clastogenic in *in vivo* assays such as the dominant lethal, cytogenetic, and micronucleus assays. There is no evidence to suggest that MMA is genotoxic to man.

The results of thorough epidemiological studies are consistent with laboratory studies showing a lack of genotoxic or carcinogenic activity in experimental animals exposed to MMA. Epidemiology studies of workers exposed to MMA showed no excess of respiratory disease. In a retrospective mortality study conducted in a plant using MMA, ethyl acrylate, and other chemicals to make acrylic sheet, an excess of colon cancer was reported among the workers exposed more than 40 years ago. Several follow-up cohort mortality, case-control, and other epidemiological studies did not reveal an increased risk for colorectal or other cancer from MMA. The International Agency for Research on Cancer (IARC) recently concluded there is inadequate evidence for carcinogenicity of MMA in humans, and concluded the evidence suggests lack of carcinogenicity of MMA in experimental animals (IARC, 1994). Thus, MMA does not present a carcinogenic hazard to man.

Methyl methacrylate was not teratogenic in several animal experiments using unusual protocols or routes of exposure, although maternal toxicity, delayed ossification and some indication of embryonic death at high levels were noted. Although these early developmental toxicity studies showed no compelling evidence that MMA is a developmental hazard at maternally non-toxic levels, most reviewers cite a study by Singh (1972), who administered MMA by intraperitoneal injection to groups of five pregnant rats, showing increased hemangiomas in offspring.

The relevance of these early studies is difficult to judge due to problems with experimental design (few animals, various treatment schedules and routes, lack of maternal toxicity data, excessively high exposure level, and inconsistent results), and these data are insufficient to assess MMA's developmental hazard potential. In a later, well-conducted study of rodents exposed by the inhalation route at concentrations up to 2028 ppm, MMA was not embryofetotoxic or teratogenic, even at maternally toxic dose concentrations (Solomon et al., 1993). By virtue of its lack of teratogenicity and lack of effect on reproductive organs in several lifetime exposure studies, and negative results in a dominant lethal study, MMA is not expected to have an adverse effect on reproduction or fertility. No reproductive or teratogenic effects of MMA in exposed populations have been reported.

Methyl methacrylate is not considered to present a risk to the environment based on its low aquatic toxicity, low bioaccumulation potential, volatility and biodegradability in the aquatic environment under aerobic conditions. Methyl methacrylate is classified as slightly to essentially non-toxic to fish and other aquatic organisms (protozoa, microcrustacea, algae). Methyl methacrylate is not expected to bioaccumulate and there is no evidence of long-term adverse effects.

Based on fugacity modeling, the majority of MMA released to the environment is expected to enter the atmospheric compartment. Its atmospheric half-life is estimated at 3 hours. Although it is not considered readily biodegradable according to OECD criteria, MMA is inherently biodegradable with 88% degraded within 28 days in the aquatic environment under aerobic conditions. In soil, MMA does not adsorb to organics in most soil types, but is expected either to rapidly evaporate or to biodegrade. Methyl methacrylate is not considered to be a significant environmental hazard.

In conclusion, MMA is a weak skin sensitizer and an irritant to the skin, eyes, and the respiratory tract. Experience in man suggest there is no clear evidence that MMA is hazardous during manufacture or use in a number of industries. No genotoxic, carcinogenic, reproductive or teratogenic effects have been reported in exposed populations. The pungent odor and irritant nature of MMA serves as a warning property and tends to keep inhalation and dermal exposure low. These observations agree with the lack of genotoxic, carcinogenic, reproductive or teratogenic effects observed in laboratory studies on MMA. Environmental fate and aquatic toxicity studies suggest MMA does not represent an environmental hazard. Based on the large database, the likelihood of any other short- or long-term health effects to man or the environment is limited.

Exhibit C

EPA Draft IRIS Support Document, "Toxicological Review: Methyl Methacrylate" (Feb. 10, 1997)

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Exhibit D

Applying a Physiologically Based Deposition Model for Methyl Methacrylate in the Olfactory Regions of the Rat and Human Nose to Estimate Dosimetric Adjustment Factors

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Report Summary: A PB-PK model of the nasal deposition of methyl methacrylate (MMA) has been used to predict the amount of MMA hydrolyzed in target nasal olfactory tissues during inhalation exposures. The model has three nasal regions, each of which contains an air compartment, a mucus layer, an epithelial tissue compartment, and a blood exchange region. Anatomical and air flow characteristics were obtained from previously developed models, partition coefficients were estimated based on analogy with values derived for other esters and diffusivity constants were fit based on the observed uptake of non-metabolized vapors. Estimates of metabolic parameters for MMA hydrolysis were derived from studies with MMA itself and by analogy with results on the enzyme distribution between species obtained with vinyl acetate (VA). Deposition studies with MMA reported by Morris and Frederick (1995) were then used to refine the rat metabolic parameters. This calibrated model was used to calculate the olfactory epithelial tissue exposures in rats and humans exposed for 6 hours to a concentration equivalent to the Benchmark Dose Concentration (28.4 ppm) derived from the lifetime toxicity studies. The ratio of these tissue doses provides an estimate of the appropriate dosimetric adjustment factor (DAF) to be applied in arriving at a human equivalent concentration. The DAFs obtained with the MMA model were between 0.64 and 0.67, depending on whether metabolic parameters were obtained from studies with MMA or from studies with VA. These estimates were made based on several health conservative assumptions. In general, a DAF of about 1.0 is supported by the modeling results and by considering differences in breathing patterns and gas-tissue equilibration within the nasal cavities of rats and humans. Sensitivity analysis conducted for the rat showed that the deposition in rats is controlled more by flow and diffusivity than by metabolism. In the human simulations, metabolic parameters, because they are smaller than in the rat, actually are more important. This PB-PK model for nasal dosimetry of MMA should be important in extending the present RfC methodology to explicitly consider regional deposition metabolism at specific sites within the nose.

Introduction:

Methyl methacrylate (MMA) is a clear, flammable liquid with a strong odor. It has a low degree of acute toxicity and is mildly irritating to the eyes and respiratory tract. Although it is a skin sensitizer, its sensitization potential is very low. In chronic inhalation studies, toxic effects were restricted to the upper respiratory tract, where degeneration of the nasal epithelium was observed at the higher concentrations, 100 and 400 ppm. In this chronic study, the No Observed Adverse Effect Level (NOAEL) for nasal effects was 25 ppm. Other esters, including ethyl acrylate (EA) and vinyl acetate (VA) also cause lesions in the nasal olfactory epithelium. These esters are hydrolyzed by carboxylesterase enzymes in nasal tissues to their corresponding acids and alcohols or, as with VA, to an acid and aldehyde product. The toxicity of these esters in the nasal olfactory epithelium is believed to predominantly arise from the irritant properties of the metabolites released on parent compound hydrolysis.

Reference Concentrations (RfCs) are usually established based on the NOAEL or on a statistically derived response measure, the LED10 or Benchmark Dose Concentration (BMD). These values are next adjusted for duration to equate the effect to continuous exposure (ADJ1) and for interspecies differences in respiratory tract dosimetry using a dosimetric adjustment factor (DAF). As illustrated in equation (1), these two corrections are sometimes referred to as "above the line" adjustments. Uncertainty factors (UFs) are also used to account for the adequacy of the total data base, inter-individual variability, interspecies differences, and other factors. As shown in the generic equation, these terms are sometimes referred to as "below the line" adjustments.

$$RfC = \frac{BMD\ (or\ NOAEL)\ *\ ADJ1*DAF}{UF1*UF2*UF3.....} \qquad (1)$$

Development of these RfC values for risk assessment for the effects of inhaled compounds on human health relies upon several extrapolations, notably across species, concentrations and exposure patterns. Several options may be utilized to address these

extrapolations depending upon the information available. The options related to dosimetry include default methods described for RfCs (U.S. EPA 1994). The DAF default methodology is based on general characteristics of deposition of particulate and gaseous materials in different regions of the respiratory tract. Different defaults exist for three regions - the upper respiratory tract (including the nasal structures), the tracheo-bronchial region, and the alveolar region in the deep lung. Highly reactive gases, called category I gases and vapors, react with tissues at the first site of contact in the nose, buccal cavity, or upper portions of the respiratory tract. The default for these gases is based on average deposition within the cavities. For nasal effects, average deposition is the ratio of the ventilation rate in the extrathoracic (ET) region divided by surface area (V_{ET}/SA_{ET}). In deriving a human equivalent concentration, the ratio for the animal (subscript, A) is divided by the same ratio for the human (subscript, H).

$$RfC = \frac{BMD\ (or\ NOAEL) * ADJ1 * (V_E/SA_{ET})_A/(V_E/SA_{ET})_H}{UF1*UF2*UF3....} \qquad (2)$$

These default methods were developed to account for differences in respiratory tract geometry and regional air flow across species. Such differences strongly affect chemical deposition and the dose delivered to individual sites within the respiratory tract. In addition, the guidelines describe different default options for defined classes of chemicals. These classes reflect differences between vapors and particles and between local respiratory tract toxicity and internal effects due to the absorbed chemical. One major area left unaddressed by these default options is differences in the metabolism of chemicals in the respiratory tract.

The toxicity of many compounds in the nasal cavity is specific to particular regions in the nose and depends on the metabolism of the compound in these regions. For these compounds, the process of establishing an RfC can be enhanced by applying compound specific data related to regional dosimetry in the nose. Several physiologically based pharmacokinetic (PB-PK) models are now available for evaluating the factors important in nasal dosimetry and for improving the scientific basis of the dosimetric adjustment strategies

for metabolized compounds. Nasal dosimetry modeling efforts have been reported for several esters, including ethyl acetate (Morris *et al.* 1993) and vinyl acetate (Plowchalk *et al.*, 1996).

Methods:

***PB-PK Model Structures*:** A generalized PB-PK model for nasal uptake and metabolism of vapors was developed and applied to a series of five chemicals including ethyl acetate (Morris *et al.* 1993). This model provides a tool to quantify the importance of several factors in nasal deposition. Regional airflow, tissue distribution of the respiratory and olfactory mucosa and regional distribution of metabolizing enzymes are all described in the model. Airflow was broken up into two pathways, a dorsal medial pathway representing 8% of airflow and the lateral ventral pathway (92%) representing the remainder of air flow (i.e. flow through the dorsal lateral meatus, middle meatuses, ventral lateral meatus, and ventral medial meatus). The dorsal medial flow passes over both respiratory and olfactory epithelia, while the lateral ventral flow only passes over respiratory epithelia.

In the Morris model, the nasal tissues are further divided into stacks of subcompartments for the mucus, epithelium, and submucosa. These subcompartments provide a simplified description of tissue anatomy. A thin layer of mucus (10 μm) on the surface of the tissue is assumed to be equilibrated with the air flowing past. The tissues themselves are also broken down into 10 uM compartments to permit short-pathlength diffusion between adjacent compartments. Intercompartmental diffusion clearances are calculated based on estimates of molecular diffusivity, surface areas, and diffusion path lengths. Blood equilibrates with the submucosal compartment and unreacted parent chemical in this bottom tissue compartment passes into the blood and into the systemic circulation. It is unlikely that concentrations in these stacks can ever be determined experimentally during actual exposures. At the present time, the available data base for model development with MMA and most other gases is limited to measurements of total extraction of these compounds from the airstream.

The distribution of metabolic enzymes is described for the respiratory and olfactory mucosal subcompartments based upon immunohistochemical and other measurements. As illustrated by Morris (1993) for the various enzymes important for the five substrates considered, the distribution varies greatly between tissue types within the nasal cavity. This metabolizing capacity plays the major role in the uptake of these chemicals in the nose by removing parent chemical and altering the mass balance, thereby increasing diffusion of parent compound into the tissue. This role of metabolism in regulating deposition of esters has been demonstrated for methyl methacrylate and ethyl acrylate (Morris and Frederick, 1995) both by model simulation and experimentally by collecting data in animals treated with BNPP, bis-nitrophenylphosphate, a specific inhibitor of carboxylesterases.

Although the model of Morris *et al.* (1993) only described the nose, the effluent air could easily be linked to models for the remainder of the body. Frederick *et al.* (1995) discussed a model for EA which linked the effluent air from the nasal cavity to a lung equation in a generic PB-PK model for the body. The distribution and metabolism in the body were described following approaches reported by Ramsey and Andersen (1984) with styrene. With styrene and other volatile hydrocarbons, metabolism was assumed to occur only in the liver. For the esters, metabolism takes place in most organs and loss terms are provided for hydrolysis in each tissue in the PB-PK model for the body.

A recent expansion of these models has been reported for vinyl acetate (Plowchalk *et al.* 1996). This model describes the metabolism of vinyl acetate by carboxylesterases of the respiratory and olfactory mucosa to form acetaldehyde and acetic acid. The acetic acid then enters biosynthetic pathways through the formation of acetyl CoA. Olfactory mucosal toxicity from vinyl acetate is ascribed to the susceptibility of this tissue to increased concentrations of intracellular H^+-ion concentrations (i.e., reduced pH) associated with formation of acetic acid. Therefore, a submodel was developed which described the intracellular hydrogen ion concentration and the pH changes.

MMA Model Development: In the present model, the nasal cavity (Figure 1) is broken down into the same three regions as described by Morris, *et al.* (1993) - a dorsal medial

path consisting of 12% of total flow passing over respiratory and olfactory epithelium in series and a lateral ventral path representing the remaining flow passing over the majority of the respiratory epithelium. Model parameters are provided in Table 1. In each tissue region there are four subcompartments - an air phase, a mucus layer, an epithelial tissue compartment, and a blood exchange tissue compartment largely representing submucosal tissue. The mucus and tissue compartments are described with respect to surface area, tissue depths, and average diffusion distances between each compartment. The model code, written in ACSL (Advanced Continuous Simulation Language-Mitchell & Gauthier, Concord, MA) is provided in Appendix I. Electronic and hardcopy output of the model and command file are attached to the report. The operations of the models in reproducing situations and calculations reported in this paper are included in the text of the command file - **EPAMMA.CMD**.

A representative equation for the rate of change of the amount of MMA in the dorsal medial region respiratory epithelium (RDOE) is :

RDOE = KOMDD*(CDOM-CDOE)-KOEDD*(CDOE-CDOX)-RAMDOE (3)

KOMDD and KOEDD are intercompartmental clearance terms (cm^3/hr), calculated as product of the diffusivity constant times the surface area divided by the diffusional distance, for mucus to epithelial tissue and from epithelial tissue to the blood-exchange portion of the tissue, respectively. RAMDOE is the rate of metabolism of MMA in the respiratory epithelial tissues and CDOM, CDOE, and CDOX, are respectively the free MMA concentrations in the mucus, epithelial tissue, and blood-exchange portions of the tissues. Metabolism is adjusted based on the volume of tissue. For instance, in equation (3).

RADMOE = VDOE*CDOE*VHOLF/(CDOE+KHD) (4)

VDOE is the volume of the dorsal olfactory epithelial compartment, VHOLF is the maximal rate of metabolism in the olfactory tissue per gram tissue, and KHD is the apparent affinity constant of the esterase for MMA.

The Air Compartment: In the Morris model all chemical entering a nasal region equilibrates with the mucus. In effect, this structure describes a case where uptake into mucus from the air phase is air-flow limited. In our MMA model, the air uptake has been reorganized to allow the possibility that diffusional barriers (equivalent to gas phase resistance) may limit availability of vapors to surface structures in various regions. The three air compartments now have a diffusional clearance term, PAi, included. The equation for **RDRWI**, the net rate of uptake (umole/hr) from the dorsal ventral air phase into respiratory mucus, is determined by air flow (DM), a diffusional clearance (PA1) and the difference in concentration between the air phase, CNCAV, and the free mucus concentration, CDRM/PMA. Thus,

$$\mathbf{RDRWI = DM*PA1/(PA1+DM)*(CNCAV-CDRM/PMA)} \qquad (5)$$

The free mucus concentration is obtained by dividing the mucus air partition coefficient (PMA) into the mucus concentration (CDRM). The effluent concentration from this region is calculated from the fraction of flow that passed through the region without equilibrating with mucus and the mucus effluent concentration from the equilibrated portion of flow. As shown in Figure 1, the exiting concentration is C2, which is calculated from equation (6):

$$\mathbf{C2 = CDRM/PMA*PA1/(PA1+DM) + CNCAV*DM/(DM+PA1)} \qquad (6)$$

For each air compartment a PAiC term is provided. The units of this term are multiples of the air-flow through the compartment. PA1 is calculated by multiplying DM by PA1C. The default values used with both rats and humans were PACs of 10. When PAC is 10, over 90% of the incoming mass is available for uptake, i.e., 100*(PA1/(PA1+DM)) is about 91%.

Tissue metabolism: Tissue clearance of MMA occurs due to hydrolysis catalyzed by carboxylesterase enzymes throughout the body. Based on previous studies with these esters (Frederick, personal communication) the concentration of these enzymes in the nasal

regions can be related to their concentration in liver. On a per gram tissue basis in the rat, the respiratory activity is about 31% of liver; the olfactory activity is about 83% of liver. In olfactory tissue the majority of the activity has been associated with sub-mucosal (blood exchange) tissues and Bowman's Glands in this region. In the model we apportioned the olfactory tissue activity between the epithelium and the sub-mucosal blood exchange region to give average olfactory activity equal to the liver. These calculations were based on the results of Bogdanffy *et al.* (1995) for the distribution of esterase activity toward VA in these tissues. All esterase activity in the respiratory tissue regions was placed in the epithelial compartment. With EA, the models for tissue metabolism include terms for conjugation with glutathione and direct reactivity with tissue nucleophiles (Frederick *et al.*, 1992). Because, the second-order rates of reaction of MMA with GSH are only about 1.2% as large as that for EA (McCarthy *et al.*, 1994), terms for MMA reaction with GSH or with other tissue nucleophiles were not included in this MMA model.

Partition Coefficients: The partition coefficients for MMA have not been determined directly. Blood:air partition coefficients have been determined for ethyl acetate (74), vinyl acetate (29), and ethyl acrylate (100). The mucus-air partition coefficient has been assumed to be similar to blood-air partition coefficient. Tanii and Hashimoto (1982) report the log of the octanol-water partition coefficients for ethyl acrylate and MMA. The values are 1.33 and 1.38, respectively. These values are equivalent to octanol-water partition coefficients of 21.3 and 24.0 for the two compounds. Due to the similarities in octanol water partition coefficients and in water solubility of MMA and EA, the partitions for EA were used as starting values for MMA. Final values providing good representations of the extraction data were a mucus-air partition coefficient (PMA) equal to 75.

Tissues: Blood flows for the rat were expressed as proportion of cardiac output and were obtained from the work of Delp *et al.* (1991). The proportion of total blood flow perfusing the upper respiratory tract was estimated to be 0.005 (Stott *et al.*, 1986). Volumes for skin, muscle, fat, richly perfused tissues, venous blood, and arterial blood are from Delp *et al.* (1991).

Output Variables: The model was calibrated against the deposition studies reported for MMA by Morris and Frederick (1995). These experiments estimated the upper respiratory tract deposition (**URTDEP**) of MMA in the nose. In the model **URTDEP** is calculated based on the incoming and effluent concentrations:

$$\mathbf{URTDEP = (CNCAV-(DM*C3+RM*C1)/INHAL)/CNCAV} \qquad (7)$$

URTDEP is equivalent to the extraction of vapors from the airstreams passing through the nose.

Simulations: For data evaluation, a concentration response model was constructed to permit re-iterative simulations with plots of the steady-state extraction expected at the end of the one hour experiment. In this mode, the model is run for a single concentration for one hour. The end of exposure values are tabulated with new names. For instance, at the end of the run, **ppm** is stored as **pppm**, **urtdep** is stored as **purtdep**, and tissue dose in the olfactory epithelium, **odose**, is stored as **podose**. The model operations are controlled by the terminal block and a restart in the initial section. The variable changing each time through the model is the inhaled concentration, **ppm**. The terminal block tests **ppm** to see if it has reached a maximum value, **ppmmax**. If the maximum is reached, the program terminates. If it has not been reached, control returns to restart; the concentration is incremented by a value ppmdelt, and the model exercised again. After termination of the program, the results of many individual runs are plotted in a concentration-response plot. (See, for instance, Figure 2.) The curve in Figure 2 was generated for **tstop** = 1 hr, **ppmmin** = 1 ppm, **ppmdelt** = 25 ppm, and **ppmmax** = 600 ppm. The independent variable in the plot, **pppm**, is the concentration during individual runs. For the nasal uptake studies a model constant '**MORRIS**' is set to 1.0. This diverts the effluent air away from the lungs and the concentration of MMA in the inhaled air entering the lungs via the trachea is 0.0. For whole body simulations, '**MORRIS**' is set to 0.0. In this situation, the effluent nasal air containing MMA enters the lung and is available for equilibration in the alveolar region of the respiratory tract. When **MORRIS** is 0.0, the mass balance on the model can be checked by insuring that the amount inhaled (**AINHAL**) is equal to total mass (**TMASS**).

The model is also easily run for a single concentration, such as in calculation of the values of the olfactory tissue dosimeter- **odose**, by setting **ppmmax** to the same value as **ppmmin**. In these simulations, values of state variables and calculated parameters in the model can be plotted against time (**t**) as the independent variable as long as **t** is one of the parameters in the **prepare** statement in the command file or is prepared at run time from the command line in ACSL.

Tissue Dose Surrogates: The goal of developing this PB-PK model for MMA is calculation of DAFs for use in the RfC calculation as part of the IRIS pilot project. The limiting toxicity with MMA in chronic exposures were nasal lesions in the olfactory region of the nose. The NOAEL for these effects was 25 ppm. Minimal changes were seen at 100 ppm. A BMD was estimated by B. Allen, ICF Kaiser Engineers, Inc., Research Triangle Park, NC, of 28.4 ppm. The toxicity of MMA and other esters in the olfactory region is believed to be due to hydrolysis of the MMA to methacrylic acid which acts as a cellular irritant at high rates of production. In the model, a dose surrogate for olfactory epithelial degeneration was created based on the total production of acid in the 6 hour exposure divided by the tissue volume. The surrogate is **ODOSE** (olfactory tissue dose):

ODOSE = (Total Acid Formed in 6-hr) / (olfactory epithelial tissue volume) (8)

ODOSE is calculated for 6 hour exposures in the rat and for equivalent 6 hour exposures in humans with model parameters set in the procedural **HUMAN1** in the command file **EPAMMA.CMD**.

Sensitivity Coefficients: As with other nasal uptake models developed to date, this MMA model also has a large number of parameters (Table 1). Many of these parameters have been established by specific experiments on blood flow, on careful dissection of relevant nasal tissues, on airflow modeling using casts or by airflow simulation with super-computers, and on hydrolysis rates in various tissues. Some of the parameters, including the diffusivity constants, are first approximations that must be refined by fitting the model to

the extraction data. The importance of these various parameters for nasal deposition or for olfactory dosimetry varies with concentration and with the particular compounds, i.e., with the values of the compound specific parameters.

Sensitivity analysis can be performed to assess the relative importance of individual parameters in producing any specific outcome in the model. The sensitivity analysis with the MMA model was performed at the NOAEL, 25 ppm. The objective functions investigated were upper respiratory tract deposition (**URTDEP**) and olfactory epithelial tissue dose (**ODOSE**). In conducting the sensitivity analysis, the objective function was first estimated for the base case; then the test parameter was increased by 1% and the model exercised again. The difference in values for the two runs is divided by the original value and the quotient multiplied by 100. This value is the normalized sensitivity coefficient. A sensitivity coefficient of 1.0 means that for a 1% change in the parameter there is a 1% change in the objective function being evaluated.

Results:

Nasal Deposition Modeling: The overall uptake of MMA is complexly dependent on a series of parameters and our confidence in these parameter values is not uniform. The blood flow to the nasal structures and the distribution of epithelial tissues are now known with some precision. The tissue diffusivities in the model have to be derived by curve fitting to deposition data with a poorly metabolized vapor. With the anatomic and flow parameters set to measured values, the values for **kmuc, kolf, and kres** were adjusted to be consistent with a deposition fraction of acetone at three flow rates as reported in Morris *et al.* (1993). With the **EPAMMA** model, the acetone data are run by typing **acetone** from the command line and comparing the simulated deposition with the published results for that deposition, 0.40, 0.27, and 0.11. These results are best represented with diffusivity constants of 0.01 cm^2/hr. This value was then used with the MMA deposition studies as the value for all three diffusivity parameters.

Metabolic constants have recently been reported for MMA in microsomal preparations obtained from liver and nasal tissues by Central Toxicology Laboratory (CTL), Aderly Park, Macclesfield, Cheshire, UK. We used initial estimates of Km for MMA from this work (0.14 mM) and estimates of hepatic Vml for esters from work with ethyl acrylate conducted at Rohm & Haas (16 umol/g/min). This rate was then apportioned to olfactory and respiratory tissue, based on the ratios of activities estimated from the CTL studies. Olfactory tissue to liver ratios were 38.6/46.5 (0.83) and the respiratory tissue to liver ratio was 14.3/46.5 (0.31). The total olfactory activity per gram tissue in the rat was equal to 0.83 times the liver Vml, i.e., **FACTOR1** in the model was 0.83. **FACTOR2** was 0.31. Initially, the Km was set to 0.14 mM consistent with estimates of this constant in liver tissue by CTL. With these parameters, the deposition curve was too shallow and never attained values as great as observed in the studies themselves (Figure 2). To provide a steeper gradient in the lower concentration region of the concentration-response plot of Figure 2, the affinity constants for metabolism in the nasal epithelial compartments were decreased from 0.14 umole/ml to 0.10 umol/ml.

The sensitivity analysis for the rat simulations (Table 2) showed that the most important parameters with a negative affect on **URTDEP** in the rat studies were total flow, the proportion flow into the dorsal meatus and the affinity of the respiratory carboxylestrase for MMA. The mucus diffusivity, the surface area of the respiratory epithelium, the surface area of the olfactory epithelium, the mucus air partition coefficient, and the Vmax for the respiratory epithelium (shown by **VML** and **FACTOR2**) had strong positive correlation with deposition. In general, flow, the diffusivity constant from mucus into tissue, and the metabolic and structural characteristics of the respiratory epithelium played the largest role in fractional deposition.

Estimating Target Tissue Exposures: Olfactory tissue dose (**ODOSE**) is also shown for multiple exposure concentrations for both the human and the rat (Figure 3). In the concentration range of interest for toxicity studies, the dose surrogate increases linearly with exposure concentration. Because toxicity is occurring in a region of the nose which receives only 12% of total airflow, **ODOSE** is sensitive to a different set of parameters and

sensitive to a different degree than is **URTDEP** (Table 2). The sensitivity analysis with **ODOSE** assesses the parameters that need to be known with confidence in rats and humans to improve confidence in the PB-PK approach for estimating the DAFs.

For **ODOSE**, total flow, mucus diffusivity, portion of flow in dorsal meatus and mucus:air partition coefficient had large positive sensitivity coefficients. In contrast, the surface area of the olfactory epithelium and depth of the olfactory epithelium (both of which contribute to estimates of olfactory epithelium volume) had large negative coefficients. The affinity constant for metabolism in the olfactory epithelium had a small negative impact and the metabolism, associated with the product of **VML** and **FACTOR1,** had a small positive impact. As with **URTDEP**, the sensitivity analysis indicates that the tissue dose from MMA in the rat is more associated with flow and geometry characteristics than with the metabolic parameters.

The tissue dose at the BMD derived from the chronic study is calculated by running the model at 28.4 ppm for 6 hours. Typing **dosimeters** from the command line runs the model for rats and humans and outputs the tissue concentrations for several flow conditions. For the rat the tissue dose is 70 umol metabolized/g tissue in the 6 hour exposure with a flow rate of 197 ml/min.

Human Simulations: The parameter set for humans is provided in Table 1. The anatomical and flow characteristics and flow patterns are established from dissection and modeling experiments. The diffusivities and partition coefficients were used as estimated from the rat model above. Metabolic constants for the humans were adjusted following two different procedures.

Approach 1 used estimates of metabolic constants in nasal tissues reported by Bogdanffy *et al* (1995) in their 'Draft Working Paper for External Peer Group Review'. In their Table 28, they provide values for surface area and maximum activities for each epithelial compartment in rats and humans. For each epithelial compartment, we calculated

the ratio as Vmax per surface area. For olfactory these ratios are 5.73 (39/6.8) in the rat and 2.23 (27.9/12.5) in the human. The human/rat ratio is 0.39. Similar calculations for the respiratory region gives a human to rat ratio of 0.15. These ratios were used to adjust **FACTOR1** and **FACTOR2**, terms that calculate the activities in the olfactory and respiratory regions, respectively. The tissue/liver ratios in the rat obtained from the CTL studies, 0.83 and 0.31 were multiplied by 0.39 and 0.15, respectively, giving 0.32 (**FACTOR1**) and 0.0465 (**FACTOR2**). This parameterization of the metabolic parameters was run at a flow rate of 13,800 ml/min, based on standard US EPA values. Based on Bogdanffy *et al.* (1995) the tissue-blood exchange esterase activity in the human was set to 0.0. The resulting dosimeter for a 6 hour exposure to 28.4 ppm was 104 umoles/gm tissue.

Approach 2 corrected the metabolic parameters based on the study results from CTL. They report that the human activity in respiratory epithelium is 6-fold lower than in rat and the difference in rat and human in olfactory tissue is 13-fold. The latter comparison is more difficult to interpret due to the different distributions of esterases in rat and human olfactory tissues. In our rat model, esterase activity in olfactory tissue was distributed between olfactory epithelium and the submucosal/blood exchange region based on the VA results (Bogdanffy *et al.*, 1995). Following this approach, the distribution of activity to the olfactory epithelium was 39/206 and the distribution to the submucosal layer was 167/206. Thus, if we assume that the proportion of epithelial activity is the 39/206 of the total activity, the reduction in activity in the human is 1/13*206/39. This parameterization of the human model gives values of **FACTOR1** and **FACTOR2** of 0.41 and 0.052, respectively. The dosimeter calculated for this parameter set was 113 umoles/gm. The sensitivity analysis for the human parameter set is shown in Table 3.

Discussion:

HEC Corrections: The application of a PB-PK model in estimating the DAF is an attempt to continue improving the scientific basis of risk evaluations of inhaled gases and vapors. The RfC methodologies recognize clearly that dosimetric differences between species are critically important in arriving at sound RfC values. The default conditions in the RfC focus on interspecies differences in anatomic and physiologic characteristics that alter deposition. With these esters the generic RfC approach has to be extended to recognize that metabolism in specific regions of the nasal cavity is the primary determinant of toxicity. In these cases the RfC defaults have to be refined based on knowledge of the distribution of enzymes in the nose and the differences in distribution of these activities between rats and humans. These differences can be quantitatively incorporated into PB-PK models of the nose.

In this application, we have calculated tissue dosimetry in the target tissue for nasal olfactory degeneration based on the amount of MMA metabolized during the exposures at concentrations equal to the BMD (28.4 ppm). The DAF correction is derived by comparing the dose metrics for the human and rat. In this case the ratios are in the range from 0.67 (Bogdanffy parameter set) to 0.62 (CTL parameter set). The BMD should be multiplied by these numbers to give a Human Equivalent Concentration. The range of the BMD_{HEC} for these two calculations are 19.0 to 17.6 ppm.

Other Factors: The calculations here assumed that human and rat breathing patterns were equivalent. However, the rat is an obligate nasal breather and the human is a mixed nasal-oral breather. Thus, the DAF calculated here will be conservative in overestimating the tissue dosimetry expected in humans which assumed continuous nasal breathing. The model also assumes that the air and mucus phase are well-mixed, i.e., that all the air flowing through each compartment equilibrates with the mucus in that compartment. This assumption is more likely to be accurate for the rat where there are several compounds, including vinyl acetate, where fractional deposition is nearly 1.00. It is probably less likely to be valid in humans. The model can easily be adjusted to account for poor mixing by adjusting the diffusional clearances from the air phase to make them more limiting for total uptake. PAC's in the present model were 10 times compartment air flows. As PACs are reduced, less compound is available for equilibration. If humans have lower availability than

do the rats, the estimates of human dosimetry here would be high. Because of the expected impact of nasal-oral breathing and reduced equilibration in humans, the estimates of the DAFs developed here are expected to be health conservative. With these considerations in mind, we would propose a DAF of 1.0 for MMA based on modeling and these biological considerations.

Other Factors in Estimating and RfC Value for MMA: The new cancer guidelines emphasize the central role of mode of action in determining extrapolation methodologies and the tissue dose measures that are involved in toxic actions of compounds. These DAF calculations for MMA are based on tissue acid production leading to an irritant mode of action. Knowledge of the mode of action also plays a role in assessing if other adjustments are appropriate in setting the RfC with MMA. It is not at all clear whether the adjustments for duration are necessary for compounds that act as nasal tissue irritants. Obviously, there are many factors that have to be considered in establishing an RfC for MMA. The model-based calculations of the dosimetric adjustment factor described here is only one part of a larger process.

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Figure Legends:

Figure 1: A Schematic of the Compartments in the Model for Nasal Deposition of Inhaled Methyl Methacrylate. The model consisted of 3 regions divided into 4 compartments - air, mucus, epithelium, and tissue exchange/submucosa. The total flow, INHAL, is divided into flow over through a dorsal medial pathway (DM) and through a ventral pathway (RM). The compound eventually diffuses to a blood exchange compartment where, after equilibrating with blood, it can be carried to the rest of the body in the venous blood.

Figure 2: Modeling the Dose-Dependent Disposition of MMA in the Rat Nose. Data are from Morris and Frederick (1995) for deposition with uni-directional flow at 200 ml/min. Parameters for the model are in Table 1.

Figure 3: Display of the Calculated Tissue Dose Metrics in Rats and in Humans. The tissue dose, umole metabolized per gm target tissue, is calculated for 6-hr exposures at differing exposure concentrations. The lower curve is for humans; the upper curve is for rats. The human curve was obtained with the parameter set based on the metabolic parameters obtained from Bogdanffy *et al.* (1995).

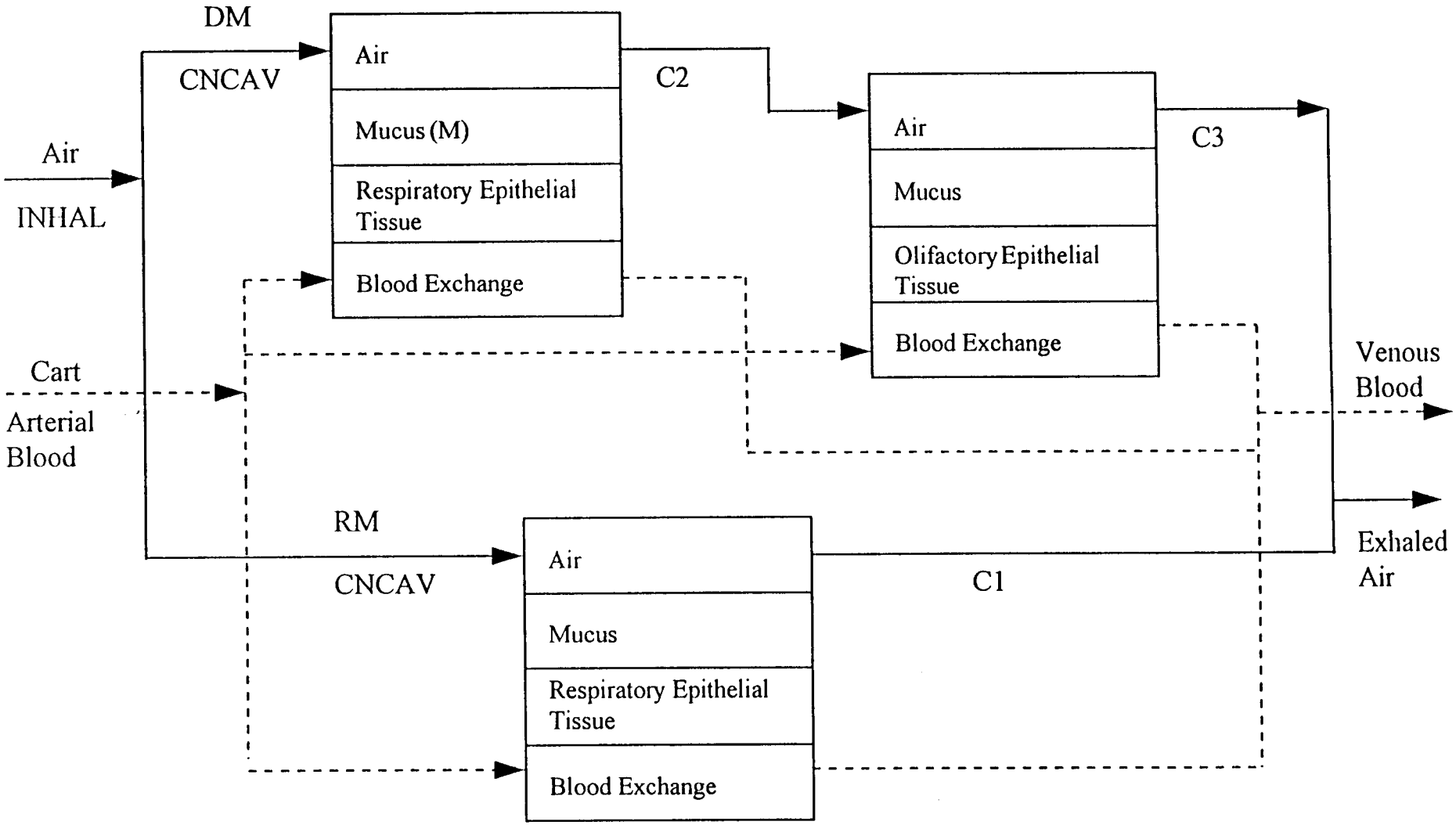
DM
CNCAV
Air
INHAL
Air
Mucus (M)
Respiratory Epithelial Tissue
Blood Exchange
C2
Air
Mucus
Olifactory Epithelial Tissue
Blood Exchange
C3
Venous Blood
Cart
Arterial Blood
RM
CNCAV
Air
Mucus
Respiratory Epithelial Tissue
Blood Exchange
C1
Exhaled Air

Figure 1:

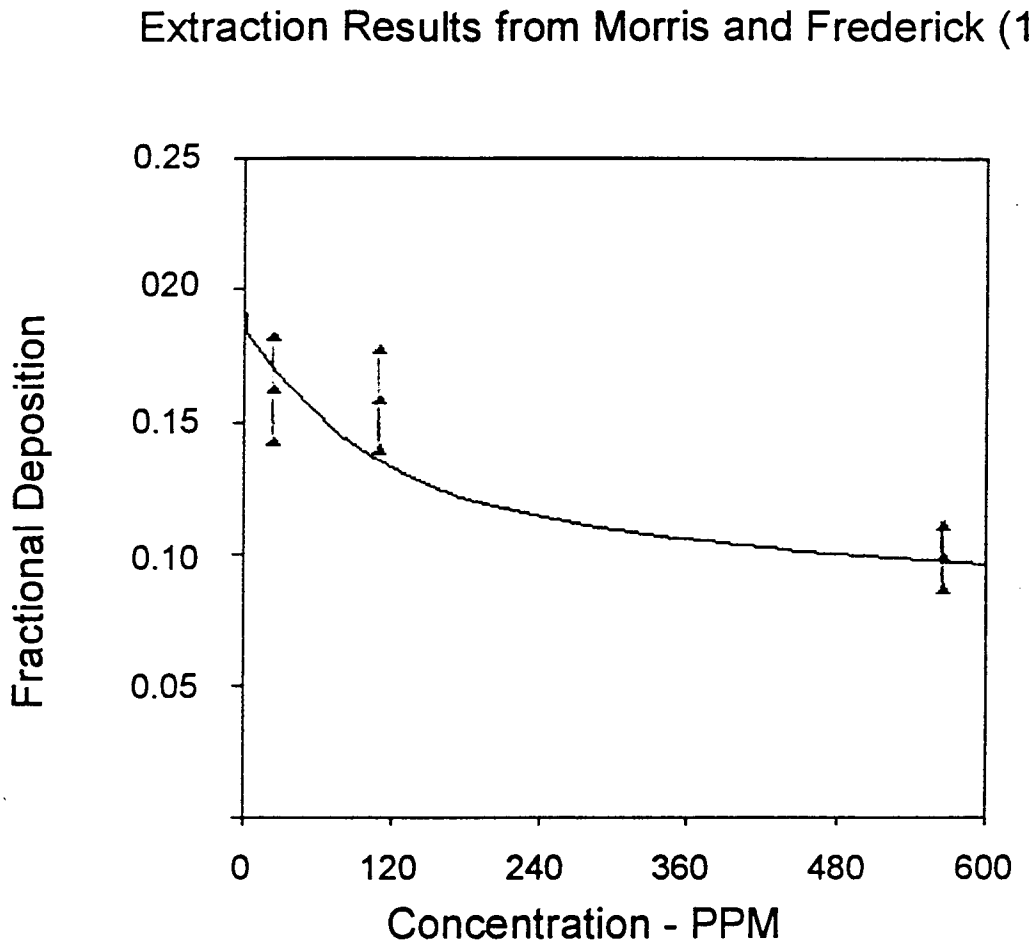
Extraction Results from Morris and Frederick (1995)
Fractional Deposition
0.25
020
0.15
0.10
0.05
0
120
240
360
480
600
Concentration - PPM

FIGURE 2:

Table 1: Model Parameters

Abbr.	Definition (units)	Rat	Human
INHAL	Flow Rate (ml/min)	200	7500
KMUC	Mucus Diffusivity (cm^2/hr)	0.01	0.01
KRES	Respiratory Diffusivity (cm^2/hr)	0.01	0.01
KOLF	Olfactory Diffusivity (cm^2/hr)	0.01	0.01
DM	Proportional Flow Dorsal Meatus	0.12	0.11
RM	Proportional Flow Ventral Path	0.88	0.89
BW	body weight (g)	200	70000
VLUC	lung volume (proportion bw)	0.004	0.014
VLC	liver volume (proportion bw)	0.035	0.026
VMFC	muscle/fat volume (proportion bw)	0.4	0.6
VRIC	rich/other volume (proportion bw)	0.407	0.2
VVBC	venous blood volume (proportion bw)	0.06	0.046
VABC	arterial blood volume (proportion bw)	0.03	0.014
SARES	surface area respiratory epithelium (cm^2)	6.23	160
SAOLF	surface area olfactory epithelium (cm^2)	6.75	12.5
FRDR	fraction respiratory epithelium in dorsal airstream	0.08	0.1
FRWR	fraction respiratory epithelium in ventral airstream	0.92	0.9
WRM	mucus depth, respiratory (cm)	0.001	0.001
WOM	mucus depth, olfactory (cm)	0.001	0.001
WRE	width of respiratory epithelial layer (cm)	0.0025	0.0025
MRE	midpoint, respiratory epithelial layer (cm)	0.00125	0.00125
WRX	width, respiratory blood exchange (cm)	0.0075	0.0075
MRX	midpoint, respiratory blood exchange (cm)	0.00375	0.00375
WOE	width, olfactory epithelial layer (cm)	0.008	0.008
MOE	midpoint, olfactory epithelial layer (cm)	0.004	0.004
WOX	width, olfactory blood exchange (cm)	0.005	0.005
MOX	midpoint, olfactory blood exchange (cm)	0.0025	0.0025

Table 1: Model Parameters (cont.)

PB	blood:air partition	50	50
PLU	lung:blood partition	1.0	1.0
PL	liver:blood partition	2.6	2.6
PMF	muscle/fat:blood partition	3.1	3.1
PRI	rich tissue:blood partition	1.0	1.0
PX	URT:blood partition	1.0	1.0
PMA	mucus:air partition	75	75
QCC	cardiac output (ml/hr-kg)	14000	14000
QLC	liver blood flow (portion QCC)	0.173	0.07
QMFC	muscle/fat blood flow (portion QCC)	0.35	0.305
QRIC	rich/other blood flow (portion QCC)	0.467	0.620
QNOSEC	URT blood flow (portion QCC)	0.01	0.005
FRESQ	portion URT flow to respiratory epithelium	0.8	0.9
FOLFQ	portion URT flow to olfactory epithelium	0.2	0.2
KHR	Km respiratory tissue (μmole/ml)	0.1	0.1
KHD	Km olfactory tissue (μmole/ml)	0.1	0.1
KHL	Km liver tissue (μmole/ml)	0.1	0.1
KHLU	Km lung tissue (μmole/ml)	0.2	0.2
KHRI	Km rich/other tissue (μmole/ml)	0.2	0.2
KHMF	Km muscle/fat tissue (μmole/ml)	0.2	0.2
VML	Liver Vmax (μmole/ml/min)	16.0	16.0
VMLU	Lung Vmax (μmole/ml/min)	3.0	3.0
VMRI	Rich/other Vmax (μmole/ml/min)	0.4	0.4
VMMF	Muscle/fat Vmax (μmole/ml/min)	0.1	0.1
PA1C	air diffusional clearance (fold - DM)	10.0	10.0
PA2C	air diffusional clearance (fold - DM)	10.0	10.0
PA3C	air diffusional clearance (fold - RM)	10.0	10.0
FACTOR1	adjusts Vmax olfactory (fold - Vml)	0.83	0.32
FACTOR2	adjusts Vmax respiratory (fold - Vml)	0.31	0.0465

Table 2 - Sensitivity Analysis for URTDEP and ODOSE at 25 ppm Using Rat Parameter Values

		URTDEP	ODOSE		
Original Values		0.1689	1.9405		
		Objective Function Values		Sensitivity Coefficients (%)	
Parameter	Values	URTDEP	ODOSE	URTDEP	ODOSE
MLMIN	200.0	0.1676	1.9503	-0.76	0.50
KMUC	0.01	0.1695	1.9491	0.37	0.45
KRES	0.01	0.1689	1.9404	0.04	0.00
KOLF	0.01	0.1689	1.9389	0.00	-0.08
DM1	0.12	0.1679	1.9503	-0.57	0.51
DMX1	0.12	0.1689	1.9405	0.00	0.00
RM1	0.88	0.1689	1.9405	0.00	0.00
BW	200.0	0.1689	1.9404	0.02	0.00
VLUC	0.004	0.1689	1.9405	0.00	0.00
VLC	0.035	0.1689	1.9405	0.00	0.00
VMFC	0.40	0.1689	1.9405	0.00	0.00
VRIC	0.407	0.1689	1.9405	0.00	0.00
VVBC	0.06	0.1689	1.9405	0.00	0.00
VABC	0.03	0.1689	1.9405	0.00	0.00
SARES	6.23	0.1699	1.9394	0.59	-0.06
SAOLF	6.75	0.1691	1.9317	0.15	-0.45
FRDR	0.05	0.1689	1.9393	-0.01	-0.06
FRWR	0.95	0.1689	1.9405	0.03	0.00
WRM	0.001	0.1689	1.9405	0.00	0.00
WOM	0.001	0.1689	1.9405	0.00	0.00
WRE	0.0025	0.1690	1.9403	0.06	-0.01
MRE	0.00125	0.1689	1.9405	0.00	0.00

Table 2 - Sensitivity Analysis for URTDEP and ODOSE at 25 ppm Using Rat Parameter Values (continued)

		URTDEP	ODOSE		
Original Values		0.1689	1.9405		
		Objective Function Values		Sensitivity Coefficients (%)	
Parameter	Values	URTDEP	ODOSE	URTDEP	ODOSE
WRX	0.0075	0.1689	1.9405	0.00	0.00
MRX	0.00375	0.1688	1.9405	-0.03	0.00
WOE	0.008	0.1687	1.9163	-0.11	-1.25
MOE	0.004	0.1689	1.9405	0.00	0.00
WOX	0.005	0.1689	1.9404	0.00	0.00
MOX	0.0025	0.1689	1.9411	0.00	0.03
PB	50.0	0.1689	1.9405	0.00	0.00
PLU	1.0	0.1689	1.9405	0.00	0.00
PL	1.0	0.1689	1.9405	0.00	0.00
PMF	3.0	0.1689	1.9405	0.00	0.00
PRI	1.0	0.1689	1.9405	0.00	0.00
PX	1.0	0.1688	1.9405	-0.03	0.00
PMA	75.0	0.1700	1.9500	0.68	0.49
QCC	14000.0	0.1689	1.9404	0.03	0.00
QLC	0.173	0.1689	1.9405	0.00	0.00
QMFC	0.35	0.1689	1.9405	0.00	0.00
QRIC	0.472	0.1689	1.9405	0.00	0.00
QNOSEC	0.005	0.1689	1.9404	0.03	0.00
FRESQ	0.80	0.1689	1.9404	0.03	0.00
FOLFQ	0.20	0.1689	1.9405	0.00	0.00
KHR	0.10	0.1685	1.9408	-0.22	0.02
KHD	0.10	0.1688	1.9375	-0.02	-0.15
KHL	0.10	0.1689	1.9405	0.00	0.00

Table 2 - Sensitivity Analysis for URTDEP and ODOSE at 25 ppm Using Rat Parameter Values (continued)

		URTDEP	ODOSE		
Original Values		0.1689	1.9405		
		Objective Function Values		Sensitivity Coefficients (%)	
Parameter	Values	URTDEP	ODOSE	URTDEP	ODOSE
KHLU	0.20	0.1689	1.9405	0.00	0.00
KHRI	0.20	0.1689	1.9405	0.00	0.00
KHMF	0.20	0.1689	1.9405	0.00	0.00
VML	16.0	0.1694	1.9431	0.32	0.13
VMLU	3.0	0.1689	1.9405	0.00	0.00
VMRI	0.40	0.1689	1.9405	0.00	0.00
VMMF	0.10	0.1689	1.9405	0.00	0.00
PA1C	10.0	0.1689	1.9405	0.00	0.00
PA2C	10.0	0.1689	1.9413	0.01	0.04
PA3C	10.0	0.1689	1.9405	0.01	0.00
FACTOR1	0.83	0.1689	1.9436	0.02	0.16
FACTOR2	0.31	0.1694	1.9399	0.31	-0.03
FACTOR3	1.0	0.1689	1.9404	0.00	0.00

Table 3 - Sensitivity Analysis for URTDEP and ODOSE at 25 ppm Using Human Parameter Values

		URTDEP	ODOSE		
Original Values		0.0523	2.4830		
		Objective Function Values		Sensitivity Coefficients (%)	
Parameter	Values	URTDEP	ODOSE	URTDEP	ODOSE
MLMIN	7500.0	0.0518	2.4848	-0.94	0.07
KMUC	0.01	0.0524	2.4987	0.24	0.63
KRES	0.01	0.0525	2.4825	0.44	-0.02
KOLF	0.01	0.0523	2.4795	0.00	-0.14
DM1	0.11	0.0512	2.4848	-2.09	0.07
DMX1	0.11	0.0523	2.4830	0.00	0.00
RM1	0.88	0.0523	2.4830	0.00	0.00
BW	70000.0	0.0523	2.4828	0.04	-0.01
VLUC	0.014	0.0523	2.4830	0.00	0.00
VLC	0.026	0.0523	2.4830	0.00	0.00
VMFC	0.60	0.0523	2.4830	0.00	0.00
VRIC	0.20	0.0523	2.4830	0.00	0.00
VVBC	0.046	0.0523	2.4830	0.00	0.00
VABC	0.014	0.0523	2.4830	0.00	0.00
SARES	160.0	0.0527	2.4820	0.84	-0.04
SAOLF	12.5	0.0523	2.4823	0.06	-0.03
FRDR	0.10	0.0523	2.4820	0.00	-0.04
FRWR	0.90	0.0523	2.4830	0.04	0.00
WRM	0.001	0.0523	2.4830	0.00	0.00
WOM	0.001	0.0523	2.4830	0.00	0.00
WRE	0.0025	0.0522	2.4831	-0.11	0.00
MRE	0.00125	0.0523	2.4830	0.00	0.00

Table 3 - Sensitivity Analysis for URTDEP and ODOSE at 25 ppm Using Human Parameters

		URTDEP	ODOSE		
Original Values		0.0523	2.4830		
		Objective Function Values		Sensitivity Coefficients (%)	
Parameter	Values	URTDEP	ODOSE	URTDEP	ODOSE
WRX	0.0075	0.0523	2.4830	0.00	0.00
MRX	0.00375	0.0521	2.4834	-0.33	0.02
WOE	0.008	0.0523	2.4561	-0.03	-1.08
MOE	0.004	0.0523	2.4830	0.00	0.00
WOX	0.005	0.0523	2.4830	0.00	0.00
MOX	0.0025	0.0523	2.4843	0.00	0.05
PB	50.0	0.0523	2.4830	0.00	0.00
PLU	1.0	0.0523	2.4830	0.00	0.00
PL	1.0	0.0523	2.4830	0.00	0.00
PMF	3.0	0.0523	2.4830	0.00	0.00
PRI	1.0	0.0523	2.4830	0.00	0.00
PX	1.0	0.0523	2.4832	-0.05	0.01
PMA	75.0	0.0528	2.5041	0.88	0.85
QCC	14000.0	0.0523	2.4828	0.05	-0.01
QLC	0.07	0.0523	2.4830	0.00	0.00
QMFC	0.30	0.0523	2.4830	0.00	0.00
QRIC	0.62	0.0523	2.4830	0.00	0.00
QNOSEC	0.01	0.0523	2.4828	0.05	-0.01
FRESQ	0.90	0.0523	2.4829	0.05	0.00
FOLFQ	0.10	0.0523	2.4828	0.00	-0.01
KHR	0.10	0.0522	2.4831	-0.13	0.01
KHD	0.10	0.0523	2.4732	-0.01	-0.39

Table 3 - Sensitivity Analysis for URTDEP and ODOSE at 25 ppm Using Human Parameters

		URTDEP	ODOSE		
Original Values		0.0523	2.4830		
		Objective Function Values		Sensitivity Coefficients (%)	
Parameter	Values	URTDEP	ODOSE	URTDEP	ODOSE
KHL	0.10	0.0523	2.4830	0.00	0.00
KHLU	0.20	0.0523	2.4830	0.00	0.00
KHRI	0.20	0.0523	2.4830	0.00	0.00
KHMF	0.20	0.0523	2.4830	0.00	0.00
VML	16.0	0.0524	2.4944	0.21	0.46
VMLU	3.0	0.0523	2.4830	0.00	0.00
VMRI	0.40	0.0523	2.4830	0.00	0.00
VMMF	0.10	0.0523	2.4830	0.00	0.00
PA1C	10.0	0.0523	2.4830	0.00	0.00
PA2C	10.0	0.0523	2.4831	0.00	0.00
PA3C	10.0	0.0523	2.4830	0.00	0.00
FACTOR1	0.32	0.0523	2.4947	0.01	0.47
FACTOR2	0.047	0.0524	2.4828	0.20	-0.01
FACTOR3	0.10	0.0523	2.4830	0.00	0.00

```
PROGRAM

INITIAL
'SORT'
'GENERAL ESTER INHALATION MODEL'
'File EPAMMA.CSL'
'Simple metabolism'
'Epithelium is not subdivided'
'Unidirectional flow'

'Model prepared by Melvin Andersen - 9/26/96'
'Based on a model provided by Dr. Clay Frederick'


'Incorporates flow dynamics data of Julie Kimbell ...
and Kevin Morgan (1992)'


'John Morris style of epithelium'
'Separates URT epithelium into three ...
compartments --- mucus, epithelium, and blood exchange layers'

'Model basically designed as described by John Morris ...
in TAP, 123, 120-129 (1993) except ...
uses Ficks law for transport and only uses one ...
blood exchange layer'

'Vapor is assumed to fully equilibrate with mucus ...
in each region'

'Metabolism in URT and all tissues'

'Divides nose into 3 compartments --- respiratory ...
region in dorsal meatus followed by olfactory ...
epithelium, and ...
and respiratory epithelium ...
on the rest of the nasal cavity'

'Uses surface area estimates for URT epithelium from ...
E.A. Gross et al., Journal of Anatomy 135, 83-88, 1982'

'Simulates John Morris deposition experiments '

'All units are expressed as ml(g), hr, or umol. in the ...
 derivative section'
```

'********** Glossary of variable names **********'

'URT = upper respiratory tract'

'R..... = Rate equation for a process (umol/hr)'
'A..... = Amount of compound from integration of a rate eqn (umol)'
'K..... = Rate constant for a reaction (/hr for 1st order process)'
'P..... = Partition coefficient (dimensionless)'
'Q..... = Blood flow (ml/hr)'
'F..... = Flow of air (ml/hr)'
'V..... = Volume (ml or cm3)'
'SA.... = Surface area (cm2)'
'D..... = Depth (cm)'
'FF.... = Fractional flow (dimensionless)'
'C..... = Concentration (umol/ml)'
'FR.... = fraction of tissue (dimensionless)'
'T..... = total of something'

'.....C = Allometric constant (Initial section only) (dimensionless)'
'.....I = Initial value'

'.D.... = Dorsal meatus region of URT'
'.S.... = Septum region of URT'
'.W.... = Lateral wall region of URT'

'..W... = Well-equilibrated air above epithelial region'
'..P... = Poorly-equilibrated air above epithelial region'
'..O... = Olfactory epithelial region'
'..R... = Respiratory epithelial region'

'.L.... = Liver'
'.LU... = Lung'
'.MF... = Muscle and fat'
'.RI... = Other perfused tissues'
'.AB... = Arterial blood'
'.VB... = Venous blood'

'...M.. = Mucus layer of URT'
'...E.. = Epithelial layer of URT'
'...X.. = Blood exchange layer of URT'
'...I.. = Flow into a compartment'
'...O.. = Flow out of a compartment'
'...P.. = Absorption rate from air into mucus'

```
'KH.... = apparent Km for carboxylesterase activity (umol/ml)'
'VH.... = apparent Vmax for carboxylesterase activity (umol/ml/hr)'


'*** Set up the model for in vivo exp or UCONN deposition exp *****'

CONSTANT RPM = 0.0 $'Resp. rate  (breaths/min)'
 RINH = RPM*60  $'Respiration rate (breaths/hr)'

CONSTANT    TVOL = 1.2 $'Tidal vol. (ml/breath)'
 MINVOL = RPM*TVOL
 QP = RINH * TVOL    $'Alveolar ventilation rate (ml/hr)'

'------------------------------------------------------------'

'Description of air flow for unidirectional experiment ...
 in John Morris lab'

'Set MLMIN=200 for simulation of a UCONN deposition experiment ...
otherwise set MLMIN=0'

CONSTANT  MLMIN = 200.0 $'Flow rate in depos. exp. (ml/min)'

FLOW = MLMIN*60  $'Flow rate (ml/hr)'

'Set INHAL = QP for in vivo exp. or = FLOW for UCONN exp'
'Units are ml/hr'

INHAL = FLOW

'Set EXHAL = INHAL for breathing and equal to 0  for UCONN exp'

EXHAL = 0.0

'In addition the concentration of the compound in the air ...
stream should be set as PPM for an in vivo experiment or ...
UGM, ug/L, for a UCONN experiment.  If PPM is set, then ...
UGM = 0 and vice versa'

' Experimental determination of nasal fractional deposition (if known)'
'Data from Morris and Frederick, Inhal. Tox., 7, 557-574 (1995)'
'Data from Stott and McKenna, Fund. Appl. Tox. 4, 594-602 (1984)'

CONSTANT REF=0.15
```

```
CONSTANT MORRIS = 1.0 $' If 1.0 simulates nasal study'

'********** Fitted values in the model ***************************'

'Diffusivity constants in each ...
epithelial region (cm2/hr), these are fitted terms in the model'

CONSTANT KMUC = 0.01 $'Diff. const. from the mucus layer ...
                      into either the respiratory or olfactory ...
                      epithelium (cm2/hr)'

CONSTANT KRES = 0.01 $'Diff. const. from the res. epi.layer ...
                into the underlying blood exchange region (cm2/hr)'

CONSTANT KOLF = 0.01 $'Diff. const. from the olf.epi.layer ...
                      of the septum into the underlying blood ...
                      exchange region (cm2/hr)'


'*** Proportional Distribution of inhaled URT Air Flow *******'

'Estimates based on modeling studies of Julie Kimbell, 1992'
'Assume that all inspired air passes over respiratory ...
epithelium and is distributed to regions of olf. epithelium ...
Values expressed as proportion of air flow (dimensionless)'

'-------------------------------------------------------------'
CONSTANT DM1 = 0.12 $'Prop. flow into dorsal meatus region ...
                      that is assumed to primarily equilibrate ...
                      with the resp. and olfactory epithelium ...
                      in the DM region and then go into the ...
                      olfactory epithelim of the ethmoid turbinates'
DM = DM1*INHAL
'-------------------------------------------------------------'
CONSTANT MM1 = 0.33  $'Prop. flow through the middle meatus ...
                    region (0.28) that is assumed to equil with ...
                    both the resp. and olfact. epithelium ...
                    across the septum plus air flow not ...
                    accounted for specifically (0.05) in air ...
                    streams'

'-------------------------------------------------------------'
CONSTANT DLVL1 = 0.42   $'Prop. flow through the dorsal and ...
                        ventral lateral meatus regions which ...
                        equilibrates only with respiratory ...
```

```
                    epithelium'

'-------------------------------------------------------------'
CONSTANT VM1 = 0.13   $'Prop. flow through the medial ...
                    ventral meatus region which ...
                    equilibrates only with respiratory ...
                    epithelium'

RM = (MM1+DLVL1+VM1)*INHAL
CONSTANT RM1 = 0.88
'-------------------------------------------------------------'

'*** Proportional Distribution of exhaled URT Air Flow *******'

'Values expressed as proportion of air flow (dimensionless)'
'Assume that air flow on exhalation follows approximately ...
the same flow pattern as on inhalation'
'-------------------------------------------------------------'
CONSTANT DMX1 = 0.12 $'Prop. flow into dorsal meatus region ...
                  that is assumed to primarily equilibrate ...
                  with the olfactory epithelium ...
                  in the DM region and then go into the ...
                  respiratory epithelium'
DMX = DMX1*EXHAL
'-------------------------------------------------------------'
CONSTANT MMX1 = 0.33  $'Prop. flow through the middle meatus ...
                region (0.28) that is assumed to equil with ...
                both the olf. and res. epithelium ...
                across the septum plus air flow not ...
                accounted for specifically (0.05) in air ...
                streams'

'-------------------------------------------------------------'
CONSTANT DLVLX1 = 0.42   $'Prop. flow through the dorsal and ...
                    ventral lateral meatus regions which ...
                    equilibrates only with respiratory ...
                    epithelium'

'-------------------------------------------------------------'
CONSTANT VMX1 = 0.13   $'Prop. flow through the medial ...
                    ventral meatus region which ...
                    equilibrates only with respiratory ...
                    epithelium'

RMX = (MMX1+DLVLX1+VMX1)*EXHAL
```

```
'-------------------------------------------------------'

CONSTANT PA1C = 10
CONSTANT PA2C = 10
CONSTANT PA3C = 10
PA1 = PA1C*DM
PA2 = PA2C*DM
PA3 = PA3C*RM

'Inhalation Modeling constants.'

CONSTANT   TSTOP =   0.5    $'Length of experiment (hr)'
 TINH = TSTOP       $'Inhalation exposure time (hr)'

'-------------------------------------------------------'



CONSTANT  PPMMIN  = 50.    $'  Inhalation dose (PPM)'
CONSTANT  PPMMAX = 1000.
CONSTANT  PPMDELT = 50.
CONSTANT PURTDEP0 = 0.20
CONSTANT    MW =  100.0   $'Molecular weight'
'-------------------------------------------------------'


'Compound-specific constants and constants determined by the ...
species being modeled.'

CONSTANT   QCC = 14000.0   $'Cardiac output (ml/hr)'

CONSTANT BW  =   200.0 $'Body wt (ml); assume 1 g = approx. 1 ml'

'********************* TISSUE VOLUMES ***********************'

        'Tissue volumes expressed as proportion body weight'

 'Measured values are from Tox. Dept. Report 87R-180'

 'Estimates for skin, muscle, fat, richly perfused tissues, ...
 venous blood, and arterial blood are based on ...
 Delp et al., Am. J. Physiol., 261, H1487-H1493, 1991'

CONSTANT   VLUC = 0.004   $' Volume of lung tissue'
```

```
CONSTANT  VLC  = 0.035   $' Volume of liver tissue'
CONSTANT  VMFC  = 0.40   $' Volume of muscle and fat tissue'
CONSTANT  VRIC  = 0.407  $' Volume of other perfused tissues'
CONSTANT  VVBC = 0.06    $' Volume of venous blood'
CONSTANT  VABC = 0.03    $' Volume of arterial blood'

'********** URT TISSUE VOLUMES **********'

'Surface area estimates and nasal cavity volume for URT from ...
E.A. Gross et al., Journal of Anatomy 135, 83-88, 1982'

CONSTANT SARES = 6.23  $'Area of URT respiratory epithelium (cm^2)'
CONSTANT SAOLF = 6.75  $'Area of URT olfactory epithelium (cm^2)'

SAET = SAOLF + SARES   $'Total surface area of res. and olf ...
                        regions (cm^2)'

'Estimates of fraction of resp. and olf. epithelium in various ...
regions of URT were made by CBF based on measurements from the ...
figures of the 4 standard URT sections in L. C. Uraih and ...
R. R. Maronpot, Env. Health Perspectives 85, 187-208, 1990'


'Respiratory'
CONSTANT FRDR = 0.05  $'Fraction in dorsal meatus (dimensionless)'
CONSTANT FRWR = 0.95  $'Fraction on lateral walls ...
                       and remaining regions (dimensionless)'

'Estimated surface area of epithelial types in various ...
regions of the URT (cm^2)'
'Respiratory'

SADR = FRDR*SARES $'Surface area of res.epi.on dor.meatus(cm^2)'
SAWR = SARES - SADR $'Surface area of res.epi.on  ...
                     remaining URT (cm^2)'

'The depth of the mucus layer in each region is based on estimates ...
by Kevin Morgan et al.'

CONSTANT  WRM = 0.001 $'Depth of mucus layer on respiratory epi.(cm)'
CONSTANT  WOM = 0.001 $'Depth of mucus layer on olfactory epi.(cm)'

'Estimates of the widths of the epithelium and blood exchange layers ...
were based measurements made from photomicrographs by CBF (1992)'
```

```
CONSTANT  WRE = 0.0025 $'Width of layer on respiratory epi.(cm)'
CONSTANT  MRE = 0.00125 $'Midpoint of resp. epi.(cm)'

CONSTANT WRX = 0.0075 $'Width of blood exchange layer under ...
                        respiratory epithelium (cm)'
CONSTANT MRX = 0.00375 $' Midpoint of blood exchange layer under ...
                        respiratory epithelium (cm)'

CONSTANT  WOE = 0.008 $'Width of layer of olfactory epi.(cm)'
CONSTANT  MOE = 0.004 $'Midpoint of layer of olfactory epi.(cm)'

CONSTANT  WOX = 0.005 $'Width of blood exchange layer under ...
                        olfactory epithelium (cm)'
CONSTANT  MOX = 0.0025 $'Midpoint of blood exchange layer under ...
                        olfactory epithelium (cm)'

'********************Volume of URT compartments***************'
                        'Olfactory'
VDOM = WOM*SAOLF $'Vol. of mucus over olf. epi (cm^3)'
VDOE = WOE*SAOLF $'Vol. of dor. meatus olf. epithelium (cm^3)'
VDOX = WOX*SAOLF $'Vol. of blood exchange region under olf ...
                    epi. of dorsal meatus (cm^3)'
VDO = VDOM+VDOE+VDOX


'Respiratory'
VDRM = WRM*SADR $'Vol. of mucus over dor. meatus res. epi (cm^3)'
VDRE = WRE*SADR $'Vol. of dor. meatus res. epithelium (cm^3)'
VDRX = WRX*SADR $'Vol. of blood exchange region under res ...
                    epi. of dorsal meatus (cm^3)'
VDR = VDRM+VDRE+VDRX

VWRM = WRM * SAWR $'Vol. of mucus over remaining res. epi (cm^3)'
VWRE = WRE * SAWR $'Vol. of remaining res. epithelium (cm^3)'
VWRX = WRX * SAWR $'Vol. of blood exchange region under res ...
                    epi.  of remaining URT (cm^3)'
VWR = VWRM+VWRE+VWRX

'*************** PARTITION COEFFICIENTS **********************'

        'Values from Toxicology Report No. 87R-180'
'Values updated based on most recent rat:blood PC determination'

CONSTANT  PB  = 50.0  $' Blood:Air Partition Coefficient'
CONSTANT  PLU = 1.0   $' Lung:Blood Partition Coefficient'
```

```
CONSTANT  PL  = 1.0  $' Liver:Blood Partition Coefficient'
CONSTANT  PMF  = 3.0  $' Muscle:Blood Partition coefficient'
CONSTANT  PRI  = 1.0  $' Other perfused:Blood Part. Coeff.'

CONSTANT  PX = 1.0  $'URT:Blood Part. Coeff.'

CONSTANT  PMA =  75.0  $' Est.Mucus:Air Partition Coefficient'

'********** TISSUE BLOOD FLOWS **********'

 'All values expressed as a proportion of cardiac output'

'Values from Delp et al. (1991)'

CONSTANT  QLC  = 0.173      $'Liver'
CONSTANT  QMFC  = 0.35       $'Muscle and fat'
CONSTANT  QRIC  = 0.467     $'Other perfused tissues'

'The proportion of cardiac output to the normal URT (0.0053) is ...
 based on the data of Stott et al. (1983) and the value of ...
 0.01 is based on the data for EA from Stott et al., (1986) ...
 which may reflect some irritation of the nasal cavity'

CONSTANT QNOSEC = 0.01

'Estimate of the vascularization of each region based on microscopic ...
examination of standard URT sections by CBF (1992)'

CONSTANT FRESQ = 0.8   $'Prop. of nasal blood flow to res. epi.'
CONSTANT FOLFQ = 0.2   $'Prop. of nasal blood flow to olf. epi.'

'The URT blood flow is divided proportionally based on the surface ...
area of each region and CBF estimate of the vascularization in ...
each region.'

QDOC = QNOSEC * FOLFQ    $'Dorsal Meatus'

QDRC = QNOSEC * FRESQ * FRDR     $'Resp. on Dorsal Meatus'
QWRC = QNOSEC * FRESQ * FRWR    $'Resp. on Remaining URT'


'********** Metabolism **********'

    ' The units of this constant are (umole/ml)'
'Values taken from preliminary data from ICI studies'
```

```
CONSTANT  KHR = 0.2   $' Est. Km for hydrolysis in Res. URT'
CONSTANT  KHD = 0.2    $' Est. Km for hydrolysis in Olf. D.M. URT'

CONSTANT  KHL  = 0.1      $' Km for hydrolysis in liver'
CONSTANT  KHLU = 0.2      $' Km for hydrolysis in lung'
CONSTANT  KHRI  = 0.2     $' Km for hydrol. in other perf.'
CONSTANT  KHMF  = 0.2     $' Km for hydrol. in muscle, fat'
CONSTANT KMU = 0.1

' The units of this constant were (umole/(ml*min)) for the ...
tissues as they were collected.'
'Values are taken as one-half EA values based on R and H ...
patent data'

CONSTANT  VML  = 16.0       $'Vmax for hydrolysis in liver'
CONSTANT  VMLU = 3.0        $'Vmax for hydrolysis in lung'
CONSTANT  VMRI = 0.4        $'Vmax for hydrol. in other perf'
CONSTANT  VMMF = 0.1        $'Vmax for hydrol. in muscle,fat'
CONSTANT  VMUC = 0.0
CONSTANT FACTOR1 = 1.00    $'Proportion of vml in olf rat'
CONSTANT FACTOR2 = 0.25    $'Proportion of vml in resp rat'
CONSTANT FACTOR3 = 1.0     $'Set at 1.0 olf rat - 0.0 olf human'

' The units of this constant are converted to (umole/(ml*hr)) ...
   for each tissue in the model.'

 VHL  = VML*60        $'Vmax for hydrolysis in liver'
 VHLU = VMLU*60    $'Vmax for hydrolysis in lung'
 VHRI = VMRI*60    $'Vmax for hydrolysis in other perf.'
 VHMF = VMMF*60    $'Vmax for hydrolysis in muscle,fat'
 VHOLF = FACTOR1*VHL       $'Vmax for hydrol. in Olf DM URT'
 VMU = VMUC*60
 VHRES = FACTOR2*VHL     $'Vmax for hydrol. in Res. URT'


'****************** END METABOLIC CONSTANTS **********************'


   'Timing commands'

CONSTANT   POINTS =  25.0  $'Number of points in plot'

ALGORITHM IALG = 2
```

```
'Plot limits'

'Exposure definition'

    CINT  = TSTOP/POINTS   $'Communication interval'

'Scaled parameters'

 BWKG = BW/1000

 QC  = QCC*BWKG**0.74

  QL  = QLC*QC
 QMF  = QMFC*QC
 QRI  = QRIC*QC

 QDR = QDRC*QC
 QWR = QWRC*QC

 QDO = QDOC*QC

'********** TISSUE VOLUMES **********'

    'Tissue volumes expressed in g = ml'
    'Assume 1 g = 1 ml of tissue volume'

  VLU = VLUC*BW
  VL  = VLC*BW
 VMF  = VMFC*BW
 VRI  = VRIC*BW
 VVB = VVBC*BW
 VAB = VABC*BW

PODOSE=0.0; PURTDEP=purtdep0; PPPM=0.0

PPM = PPMMIN - PPMDELT

RESTRT..PPM = PPM + PPMDELT


'To convert ppm to umol/ml, UMOLML'
'Conversion factor, 10 umol EA/mg'
'MGM3 = conc. in mg/m3'
MGM3 = PPM*MW/24.45
```

```
MGL=MGM3/1000
UMOLL = 10*MGL
UMOLML = UMOLL/1000

'CNCAV=Inhaled conc. of compound into the nasal cavity (umol/ml)'

CNCAV = UMOLML
TOTINH=TSTOP*CNCAV*QP

END     $'End of initial'

DYNAMIC

DERIVATIVE

'---------  Description of overall inhalation processes ----------'

'RINHAL = Rate of inhalation into the nasal cavity (umol/hr)'
RINHAL = CNCAV*INHAL
AINHAL = INTEG(RINHAL, 0.0)  $'Total inhaled (umol)'


'-----Respiratory Epithelium, Dorsal Meatus Region----------'

'RDRWI = Rate of transport into the mucus (umol/hr)'

RDRWI = DM*PA1/(PA1+DM)*(CNCAV-CDRM/PMA) - RDMMET

RDMMET = 100*VDRM*VMU*CDRM/((KMU+CDRM)*100)

 'RDRM = Rate of change of amt. of cmpd in mucus over ...
          epithelium (umol/hr)'

RDRM = RDRWI - KRMDD*(CDRM-CDRE)

KRMDD = KMUC * SADR/(WRE/2) $'Ficks 1st law diffusion constant'


 ADMMET = INTEG(RDMMET,0.0)
  ADRM = INTEG(RDRM,0.0)     $'Amount in mucus layer (umol)'
  CDRM = ADRM/VDRM           $'Conc. in mucus layer (umol/ml)'

C2 = CDRM/PMA*PA1/(PA1+DM)+CNCAV*DM/(DM+PA1)

'RDRE = Rate of change of amt. of cmpd. in res. epi. layer (umol/hr)'
```

```
RDRE=KRMDD*(CDRM-CDRE)-KREDD*(CDRE-CDRX)-RAMDRE
'KREDD = Ficks 1st law diffusion constant from  epi ...
        layer to the blood exchange region'
KREDD = KRES * SADR/((WRE/2)+MRX)


ADRE = INTEG(RDRE,0.0)     $'Amount in epithelial layer (umol)'
CDRE = ADRE/(VDRE)       $'Conc. in epithelial layer (umol/ml)'

RAMDRE = VDRE*((CDRE*VHRES)/(CDRE+KHR)) $'Rate hyd(umol/hr)'
 AMDRE = INTEG(RAMDRE,0.0)    $'Amount metab. in epithelium (umol)'


 'RDRX = Rate of change of amt. of cmpd. in blood exchange layer ...
          under respiratory  epithelium  (umol/hr)'
 RDRX = KREDD*(CDRE-CDRX)+QDR*(CA-CVDRX)
 ADRX = INTEG(RDRX,0.0) $'Amount in blood exchange layer (umol)'
 CDRX = ADRX/VDRX
 CVDRX = CDRX/PX      $'Conc. in VB leaving organ (umol/ml)'

AMDR = AMDRE

'------ Olfactory Epithelium ------'

'RDOWI = Rate of transport into the mucus (umol/hr)'

RDOWI = DM*PA2/(PA2+DM)*(C2-CDOM/PMA)

'RDOM = Rate of change of amt. of cmpd in mucus over ...
          olfactory epithelium (umol/hr)'

RDOM = RDOWI - KOMDD*(CDOM-CDOE) - RDOMMET

RDOMMET = 100*VDOM*VMU*CDOM/(100*(KMU+CDOM))

C3 = DM/(PA2+DM)*C2+PA2/(PA2+DM)*CDOM/PMA


KOMDD = KMUC * SAOLF/(WOE/2) $'Ficks 1st law diffusion constant'

 ADOMMET = INTEG(RDOMMET,0.0)
 ADOM = INTEG(RDOM,0.0)     $'Amount in mucus layer (umol)'
 CDOM = ADOM/VDOM          $'Conc. in mucus layer (umol/ml)'
```

```
'RDOE = Rate of change of amt. of cmpd. in olf. epi.  (umol/hr)'

RDOE=KOMDD*(CDOM-CDOE)-KOEDD*(CDOE-CDOX)-RAMDOE
'KOEDD = Ficks 1st law diffusion constant from  epi ...
        layer to blood exchange layer'
KOEDD = KOLF * SAOLF/((WOE/2)+MOX)
ADOE = INTEG(RDOE,0.0)     $'Amount in epithelial layer (umol)'
CDOE = ADOE/VDOE     $'Conc. in epithelial layer (umol/ml)'

RAMDOE = VDOE*((CDOE*VHOLF*0.39/1.03)/(CDOE+KHD)) $'Rate hyd(umol/hr)'
 AMDOE = INTEG(RAMDOE,0.0)    $'Amount hydrol. in epithelium (umol)'

 ODOSE = AMDOE/VDOE

 'RDOX = Rate of change of amt. of cmpd. in blood exchange layer ...
          under olfactory  epithelium  (umol/hr)'
 RDOX = KOEDD*(CDOE-CDOX)+QDO*(CA-CVDOX)-RAMDOX
 ADOX = INTEG(RDOX,0.0) $'Amount in blood exchange layer (umol)'
 CDOX = ADOX/VDOX
 CVDOX = CDOX/PX      $'Conc. in VB leaving organ (umol/ml)'

RAMDOX = VDOX*((CDOX*VHOLF*1.67/1.03*factor3)/(CDOX+KHD))
       'Rate hyd(umol/hr)'
 AMDOX = INTEG(RAMDOX,0.0)    $'Amount hydrol. in epithelium (umol)'

AMDO=AMDOE+AMDOX

'----- Remaining Respiratory Epithelium ----------'

'RWRWI = Rate of transport into the well-equil. air region (umol/hr)'

RWRWI = RM*PA3/(RM+PA3)*(CNCAV-CWRM/PMA)

'RWRM = Rate of change of amt. of cmpd in mucus over ...
          epithelium (umol/hr)'

RWRM = RWRWI - KRMWD*(CWRM-CWRE) - RDRMMET
RDRMMET = 100*VWRM*VMU*CWRM/(100*(KMU+CWRM))

KRMWD = KMUC * SAWR/(WRE/2) $'Ficks 1st law diffusion constant'

 ADRMMET = INTEG(RDRMMET,0.0)
 AWRM = INTEG(RWRM,0.0)     $'Amount in mucus layer (umol)'
 CWRM = AWRM/VWRM           $'Conc. in mucus layer (umol/ml)'
```

```
C1 = RM/(RM+PA3)*CNCAV+PA3/(PA3+RM)*CWRM/PMA

'RWRE = Rate of change of amt. of cmpd. in res. epi. layer  (umol/hr)'
RWRE=KRMWD*(CWRM-CWRE)-KREWD*(CWRE-CWRX)-RAMWRE
'KREWD = Ficks 1st law diffusion constant from  epi ...
        layer to the blood exchange region'
KREWD = KRES * SAWR/((WRE/2)+MRX)

AWRE = INTEG(RWRE,0.0)     $'Amount in epithelial layer (umol)'
CWRE = AWRE/VWRE       $'Conc. in epithelial layer (umol/ml)'

RAMWRE = VWRE*((CWRE*VHRES)/(CWRE+KHR)) $'Rate hyd(umol/hr)'
 AMWRE = INTEG(RAMWRE,0.0)    $'Amount hydrol. in epithelium (umol)'


 'RWRX = Rate of change of amt. of cmpd. in blood exchange layer ...
          under respiratory  epithelium  (umol/hr)'
 RWRX = KREWD*(CWRE-CWRX)+QWR*(CA-CVWRX)
 AWRX = INTEG(RWRX,0.0) $'Amount in blood exchange layer (umol)'
 CWRX = AWRX/VWRX
 CVWRX = CWRX/PX      $'Conc. in VB leaving organ (umol/ml)'

AMWR = AMWRE


'  ************************************************************  '

'RLUNG = Rate of delivery into the lungs (umol/hr)'
DMOUT = DM*C3
RMOUT = RM*C1
RLUNG = DMOUT + RMOUT
RLUNG1 = (1.0-MORRIS)*RLUNG
ALUNG = INTEG(RLUNG,0.0)


'==========Lungs==========='

   'RALU = Rate of change in amount in the lungs (umol/hr)'
        RALU = QC*(CV-CVLU)+RLUNG1-RAMLU-RAX
         ALU = INTEG(RALU,0.0)
         CLU = ALU/VLU
        CVLU = CLU/PLU

RAMLU = VLU*((CLU*VHLU)/(CLU+KHLU)) $'Rate of hyd. (umol/hr)'
AMLU = INTEG(RAMLU,0.0)         $'Amount hydrol. in organ (umol)'
```

```
'==========Amount Exhaled==========='

  'AX = Amount exhaled (umol)'
  CX = CLU/PB
  RAX = EXHAL*CX
   AX = INTEG(RAX,0.0)


'********** Systemic Tissue Metabolism **********'

'==========Venous Blood==========='

  'RAVB = Rate of change in amount in venous blood (umol/hr)'
   RAVB = QL*CVL+QDR*CVDRX ...
        +QDO*CVDOX+QWR*CVWRX ...
        +QRI*CVRI+QMF*CVMF-QC*CV
    AVB = INTEG(RAVB,0.0)
     CV = AVB/VVB

'==========Arterial Blood==========='

  'RAAB = Rate of change in amt. in arterial blood (umol/hr)'
  RAAB = QC*(CVLU-CA)
   AAB = INTEG(RAAB,0.0)  $' Amount in arterial blood (umol)'
    CA = AAB/VAB          $' Concentration in art. blood (umol/ml)'

'==========Liver==========='

  'AL = Amount in liver tissue (umol)'
  RAL = QL*(CA-CVL)-RAML
   AL = INTEG(RAL,0.)
   CL = AL/VL
  CVL = CL/PL


RAML = VL*((CL*VHL)/(CL+KHL)) $'Rate of hyd. (umol/hr)'

AML = INTEG(RAML,0.0)         $'Amount hydrol. in organ (umol)'

'==========Remaining Perfused Tissues==========='

  'ARI = Amount in other perfused  tissues (umol)'
  RARI = QRI*(CA-CVRI)-RAMRI
   ARI = INTEG(RARI,0.)
```

```
      CRI = ARI/VRI
      CVRI = CRI/PRI

   RAMRI = VRI*((CRI*VHRI)/(CRI+KHRI)) $'Rate of hyd. (umol/hr)'

   AMRI = INTEG(RAMRI,0.0)          $'Amount hydrol. in organ (umol)'

  '==========Muscle and Fat ==========='

      'AMF = Amount in muscle and fat tissues (umol)'
      RAMF = QMF*(CA-CVMF)-RAMMF
       AMF = INTEG(RAMF,0.)
       CMF = AMF/VMF
      CVMF = CMF/PMF

   RAMMF = VMF*((CMF*VHMF)/(CMF+KHMF)) $'Rate of hyd. (umol/hr)'
   AMMF = INTEG(RAMMF,0.0)          $'Amount hydrol. in organ (umol)'

  END      $'End of derivative'


    TERMT(T.GE.TSTOP)


   END      $'End of dynamic'
   TERMINAL

    'TMASS = mass balance (umol)'

    TMASS1 = AL+ARI+AMF+ALU+AAB+AVB+ ...
           ADRM+ADRE+ADRX+ADOM+ADOE+ ...
           ADOX+AWRM+AWRE+AWRX

    TMETAB = AML+AMLU+AMDR+ ...
           AMDO+AMWR+AMRI+AMMF+ADMMET+ADOMMET+ADRMMET

    TMASS = TMASS1+TMETAB
    'TMASS = TMASS1+TMETAB+AXOUT'

   'Check on blood flow'

   QTOT = QL+QRI+QMF+QDR+QDO+QWR

   QRECOV = 100*QTOT/QC
```

```
'RECOV = Mass balance, %'
 RECOV = 100*TMASS/AINHAL

 'URTDEP = Fract. of inhaled cmpd. deposited in URT'
URTDEP = (CNCAV -(DM*C3+RM*C1)/INHAL)/CNCAV

FLOCHK = (DM+RM) / INHAL

PODOSE = ODOSE; PURTDEP = URTDEP; PPPM = PPM
IF (PPM.LT.PPMMAX) GO TO RESTRT


END     $'End of terminal'
END     $'End of program'
```

```
'EPAMMA.CMD - PREPARED 9/23/96 BY ICF KAISER STAFF'
 'BASED ON A MODEL DEVELOPED BY CLAY FREDERICK'


'*************************************************************'

 set hvdprn=.f.
'In output statement nciout is set to points'
'This permits plotting a single value per run'

output pppm,purtdep,podose,'nciout'=500
set points=500,tstop=.2

prepare pppm, podose, purtdep

'Procedural ACETONE runs the conditions from Morris et al 1993 '
'to assess the uptake of acetone - a non-metabolized vapor from'
'the airstream.

proced acetone
set hvdprn=.f.
output 'clear' pppm purtdep ref
ace50
ace100
ace300
output 'clear' pppm purtdep podose
end

'Procedural GO runs the rat parameter set and plots the simulated'
'deposition versus the exposure concentraiton for 200 ml/min    '

proced go
rat1
set morris=1.0
set ppmmin=1 ppmdelt=25 ppmmax=600
start
pl1
end

proced pl1
plot /data=extract purtdep /char=2 /hi=0.25 /lo=0.0 /xhi=600
end

data extract (pppm,purtdep)
 23.0  0.162
```

```
 23.0  0.182
 23.0  0.142
109.0  0.158
109.0  0.139
109.0  0.177
566.0  0.098
566.0  0.110
566.0  0.086
end


'Procedure DOSIMETERS runs the model for the rat at 197 ml/min '
'at the BMD concentration (28.4 ppm).  Then it runs the 2 human'
'cases - Bogdanffy with factor1=0.32 and CTL with factor1=0.41. '
'The dosimetric adjustment factor is the ratio of the human    '
'    estimate of odose divided by the value for the rat.      '

proced dosimeters
set hvdprn=.f.
set tstop=6; rat1; set ppmmin=28.4 ppmmax=28.4 ppmdelt=.1
set mlmin=197 bw=250
set morris=0.0
start
human1; set mlmin=13800
start
set factor1=.41 factor2=.052
start
set hvdprn=.t.
end


'************************************************************'


'Set the error criteria for mucus more stringently'
'than for other tissues due to instabilities observed'
'at high extraction ratios when using a mucus pathway'

merror adom=1.e-5; xerror adom=1.e-5
merror adrm=1.e-5; xerror adrm=1.e-5
merror awrm=1.e-5; xerror awrm=1.e-5

'************************************************************'
```

```
PROCED RAT1
SET MLMIN=200      ! Flow rate ml/min
SET DM1 = 0.12     ! Proportion flow to resp/olf in dorsal meatus
SET MM1 = 0.33     ! Proportion flow through middle meatus
SET DLVL1 = 0.42   ! Proportion through dorsal/ventral meatus
SET VM1 = 0.13     ! Proportion flow through ventral meatus
SET DMX1 = 0.12    ! Proportion flow to dorsal meatus
SET MMX1 = 0.33    ! Proportion flow through middle meatus
SET DLVLX1 = 0.42  ! Proportion flow through dorsal/ventral
SET VMX1 = 0.13    ! Proportion flow through medial/ventral meatus
SET RPM = 0.0      ! Resp rate - breathes/min
SET TVOL = 1.2     ! Tidal volume - ml/breath
SET QCC = 14000    ! Cardiac output - ml/hr
SET BW = 200       ! Body weight - gm (ml)
SET VLUC = 0.004   ! Volume lung - proportion body weight
SET VLC = 0.035    ! Volume liver - proportion body weight
SET VMFC = 0.4     ! Volume muscle-fat proportion body weight
SET VRIC = 0.407   ! Volume other tissues - proportion body weight
SET VVBC = 0.06    ! Volume venous blood - proportion bw
SET VABC = 0.03    ! Volume arterial blood - proportion bw
SET SARES = 6.23   ! Surface area respiratory epithelium cm^2
SET SAOLF = 6.75   ! Surface area olfactory epithelium cm^2
SET FRDR = 0.05    ! Fraction in dorsal meatus
SET FRWR = 0.95    ! Fraction on lateral walls
SET QLC = 0.173    ! Liver blood flow - proportion total
SET QMFC =0.35     ! Muscle fat blood flow proportion total
SET QRIC =0.472    ! Rich tissue blood flow proportion total
SET QNOSEC = 0.005 ! Nasal blood flow proportion total
SET FRESQ = 0.8    ! Nasal bllod flow to respiratory mucosa
SET FOLFQ = 0.2    ! Nasal blood flow to olfactory mucosa
SET FACTOR1 = 0.83 !  Olfactory Vmax
SET FACTOR2 = 0.31 !  Respiratory Vmax
SET FACTOR3 = 1.0  ! Sustentacular/Bowman gland activity
SET MW = 100.      ! Molecular weight methyl methacrylate
SET KMUC =0.010, KRES =0.01,KOLF=0.01 ! Diffusivity cm^2/hr
SET PB=50          ! Blood air partition
SET PLU=1.0        ! Lung blood partition
SET PL=1.0         ! Liver blood partition
SET PMF=3.0        ! Muscle/fat partition
SET PRI=1.0        ! Rich/other partition
SET PX=1.0         ! URT blood partition
SET PMA=75         ! Mucus air partition
SET VMUC=0.0       ! Mucus Vmax could be included
SET VML=16.0       ! Vmax liver hydrolysis - umol/ml/min/gtissue
SET VMLU=3.0       ! Vmax lung hydrolysis
```

```
SET VMRI=0.4      ! Vmax rich tissue hydrolysis
SET VMMF=0.1      ! Vmax muscle fat tissue hydrolysis
SET KHR=0.1       ! Km resp tissue hydrolysis
SET KHD=0.1       ! Km olf tissue hydrolysis
SET KHL=0.1       ! Km liver hydrolysis
SET KHLU=0.2      ! Km lung hydrolysis
SET KHRI=0.2      ! Km rich tissue hydrolysis
SET KHMF=0.2      ! Km muscle fat tissue hydrolysis
END               ! End procedure RAT1

PROCED HUMAN1
SET MLMIN=7500
'SET KMETAB = 1.0 '
SET DM1 = 0.11
SET MM1 = 0.193
SET DLVL1 = 0.117
SET VM1 = 0.58
SET DMX1 = 0.11
SET MMX1 = 0.193
SET DLVLX1 = 0.117
SET VMX1 = 0.58
SET RPM = 0.0
SET TVOL = 750
'SET RPM = 12'
SET QCC = 14000
SET BW = 70000
SET VLUC = 0.014
SET VLC = 0.026
SET VMFC = 0.6
SET VRIC = 0.2
SET VVBC = 0.046
SET VABC = 0.014
SET SARES = 160.0
SET SAOLF = 12.5
SET FRDR = 0.1
SET FRWR = 0.9
SET QLC = 0.07
SET QMFC =0.3
SET QRIC =0.62
SET QNOSEC = 0.01
SET FRESQ = 0.9
SET FOLFQ = 0.1
SET FACTOR1 = 0.32
SET FACTOR2 = 0.0465
SET FACTOR3 = 0.0
```

```
END

PROCED ACE50
'Acetone at 50 ml/min'
RAT1
SET PMA=260
SET PB = 260.0
SET MW = 58
SET MLMIN=50
SET REF=0.4        $'Experimental data from Morris'
SET VML  = 0.0     $'Vmax for hydrolysis in liver'
SET VMLU = 0.0     $'Vmax for hydrolysis in lung'
SET VMRI = 0.0     $'Vmax for hydrol. in other perf'
SET VMMF = 0.0     $'Vmax for hydrol. in muscle,fat'
SET VMUC = 0.0
SET PPMMIN=170 PPMMAX=170 PPMDELT=0.1
SET MORRIS=1.0; START
END

PROCED ACE100
'Acetone at 100 ml/min'
SET PMA=260
SET PB = 260.0
SET MW = 58
SET MLMIN=100
SET REF=0.28       $'Experimental data from Morris'
SET VML  = 0.0     $'Vmax for hydrolysis in liver'
SET VMLU = 0.0     $'Vmax for hydrolysis in lung'
SET VMRI = 0.0     $'Vmax for hydrol. in other perf'
SET VMMF = 0.0     $'Vmax for hydrol. in muscle,fat'
SET VMUC = 0.0
SET PPMMIN=170 PPMMAX=170 PPMDELT=0.1
SET MORRIS=1.0; START
END

PROCED ACE300
'Acetone at 300 ml/min'
SET PMA=260
SET PB = 260
SET MW = 58
SET MLMIN=300
SET REF=0.11       $'Experimental data from Morris'
SET VML  = 0.0     $'Vmax for hydrolysis in liver'
SET VMLU = 0.0     $'Vmax for hydrolysis in lung'
```

```
SET VMRI = 0.0      $'Vmax for hydrol. in other perf'
SET VMMF = 0.0      $'Vmax for hydrol. in muscle,fat'
SET VMUC = 0.0
SET PPMMIN=170 PPMMAX=170 PPMDELT=0.1
SET MORRIS=1.0;START
END

PROCED XYL50
'Xylene at 50 ml/min'
SET PMA=25
SET PB = 25
SET MW = 106
SET MLMIN=50
SET REF=0.057       $'Experimental data from Morris'
SET VML  = 0.0      $'Vmax for hydrolysis in liver'
SET VMLU = 0.0      $'Vmax for hydrolysis in lung'
SET VMRI = 0.0      $'Vmax for hydrol. in other perf'
SET VMUC = 0.0
SET PPMMIN=46 PPMMAX=46 PPMDELT=0.1
END

PROCED XYL100
'Xylene at 100 ml/min'
SET PMA=25
SET PB = 25
SET MW = 106
SET MLMIN=100
SET REF=0.014       $'Experimental data from Morris'
SET VML  = 0.0      $'Vmax for hydrolysis in liver'
SET VMLU = 0.0      $'Vmax for hydrolysis in lung'
SET VMRI = 0.0      $'Vmax for hydrol. in other perf'
SET VMUC = 0.0
SET PPMMIN=46 PPMMAX=46 PPMDELT=0.1
END

PROCED XYL300
'Xylene at 300 ml/min'
SET PMA=25
SET PB = 25
SET MW = 106
SET MLMIN=300
SET REF=0.004       $'Experimental data from Morris'
SET VML  = 0.0      $'Vmax for hydrolysis in liver'
SET VMLU = 0.0      $'Vmax for hydrolysis in lung'
SET VMRI = 0.0      $'Vmax for hydrol. in other perf'
```

```
SET VMUC = 0.0
SET PPMMIN=46 PPMMAX=46 PPMDELT=0.1
END

PROCED ISO50
'Isoamyl alcohol at 50 ml/min'
SET PMA=1310
SET PB = 1310
SET MW = 88
SET MLMIN=50
SET REF=0.77        $'Experimental data from Morris'
SET VML  = 0.0      $'Vmax for hydrolysis in liver'
SET VMLU = 0.0      $'Vmax for hydrolysis in lung'
SET VMRI = 0.0      $'Vmax for hydrol. in other perf'
SET VMUC = 0.0
SET PPMMIN=27.7 PPMMAX=27.7 PPMDELT=0.1
END

PROCED ISO300
'Isoamyl alcohol at 300 ml/min'
SET PMA=1310
SET PB = 1310
SET MW = 88
SET MLMIN=300
SET REF=0.4         $'Experimental data from Morris'
SET VML  = 0.0      $'Vmax for hydrolysis in liver'
SET VMLU = 0.0      $'Vmax for hydrolysis in lung'
SET VMRI = 0.0      $'Vmax for hydrol. in other perf'
SET VMUC = 0.0
SET PPMMIN=27.7 PPMMAX=27.7 PPMDELT=0.1
END


PROCED BRB50
'Bromobenzene at 50 ml/min'
SET PMA=53
SET PB = 53
SET MW = 157
SET MLMIN=50
SET REF=0.09        $'Experimental data from Morris'
SET VML  = 0.0      $'Vmax for hydrolysis in liver'
SET VMLU = 0.0      $'Vmax for hydrolysis in lung'
SET VMRI = 0.0      $'Vmax for hydrol. in other perf'
SET VMUC = 0.0
SET PPMMIN=26. PPMMAX=26. PPMDELT=0.1
```

```
END

PROCED BRB300
'Bromobenzene at 300 ml/min'
SET PMA=53
SET PB = 53
SET MW = 157
SET MLMIN=300
SET REF=0.007        $'Experimental data from Morris'
SET VML  = 0.0       $'Vmax for hydrolysis in liver'
SET VMLU = 0.0       $'Vmax for hydrolysis in lung'
SET VMRI = 0.0       $'Vmax for hydrol. in other perf'
SET VMUC = 0.0
SET PPMMIN=26. PPMMAX=26. PPMDELT=0.1
END


'This last section sets general parameters for ACSL operation'

SET DPSITG = .TRUE.
SET CJVITG = .FALSE.
SET WESITG = .FALSE.
SET GRDCPL = .FALSE.
SET NPCCPL = 10
```

Exhibit E

SIDS MANUAL

(Second Revision)

**SCREENING INFORMATION DATA SET MANUAL
OF THE OECD PROGRAMME ON THE CO-OPERATIVE INVESTIGATION
OF HIGH PRODUCTION VOLUME CHEMICALS**

May 1996

OECD SECRETARIAT

comparison of dose-response curves for such an effect between males and females could be made both in the repeated dose toxicity and the reproduction toxicity study.

24. For the reproduction toxicity endpoint:

- when a 90-day repeated dose study is available and is sufficiently documented with respect to studying effects in the reproductive organs and a developmental study is available, the requirements for the reproduction toxicity endpoint would be satisfied;

- when either a 90-day or 28-day repeated dose study is the only repeated dose study available, it is recommended that the reproduction/developmental toxicity screening test (e.g. TG 421) be carried out in order to satisfy the requirements for the reproduction toxicity endpoint; and

- when a 90-day repeated dose study is available and demonstrates no effects on the reproductive organs, in particular the testes, then a developmental study (e.g. TG 414) can be considered as an adequate test to complete information on reproduction/developmental effect.

25. In the similar way to repeated dose toxicity, the "margin of safety" approach could be used for the initial assessment instead of using Uncertainty Factors (UF's). Guidance for the use of UF's is described in Annex 1 to this document.

26. When considering the "margin of safety" for reproduction/developmental toxicity, its magnitude can be compared to that of repeated dose toxicity. A relatively high "margin of safety" may be acceptable when severe or irreversible developmental effects occur at exposure levels below those that induce well-defined parental toxicity; in other words when the test substance produces severe developmental effects that are not secondary to general toxicity. The same level of "margin of safety" may be acceptable when developmental toxicity is only observed at exposure levels that are also toxic to the parent animals. The same considerations for the repeated dose study should be addressed when considering "margin of safety" for reproductive/developmental toxicity. An assessment of whether the effects seen in reproduction toxicity studies are secondary to general toxicity, or are specific reproductive effects, is important and expert judgement is necessary.

27. This concept of considering higher "margin of safety" for developmental effects when they occur in the absence of maternal toxicity than when observed at levels that are also toxic to the parent animals is generally accepted. However, individual Member countries may consider developmental toxicity in the presence of maternal toxicity of as great a concern as developmental toxicity seen in its absence.

4.6.6 Suggestions for Follow-up Testing

Repeated Dose Toxicity

28. If the estimated exposure level (EHE) for a specific human population is larger than or close to the estimated effect level of concern calculated from a 28-day repeated dose study, a 90-day subchronic toxicity test (TG 408, 411 or 413) could be conducted, depending on reliability of the exposure estimation. Information on exposure should be considered in detail before deciding if this is needed. In the subchronic toxicity test, a more detailed examination of effects observed in a 28-day study would be important. If the effects found in a 28-day repeated dose toxicity test are reason for concern, and substantial long-term exposure is expected to occur, it might be more effective to consider undertaking a longer toxicity test with an exposure duration of at least six months. If there is sufficient justification for a study for "conventional" (i.e. non-carcinogenic) toxic effects of longer duration than 90 days, then it should be combined with a carcinogenicity bioassay.

Exhibit F

Study Title

Inhalation Sensory Irritation (RD_{50}) Study in Mice with Selected Methacrylates and Methacrylic Acid

Author

Judith C. Stadler, Ph.D.

Study Completed on

December 20, 1993

Performing Laboratory

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P. O. Box 50
Newark, Delaware 19714

Medical Research Project No. 9498-001

Laboratory Project ID

Haskell Laboratory Report No. 615-93

INHALATION SENSORY IRRITATION (RD_{50}) STUDY IN MICE WITH SELECTED METHACRYLATES AND METHACRYLIC ACID

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to EPA TSCA (40 CFR 792) Good Laboratory Practice Standards.

Submitter: Methacrylate Producers Association

Sponsor: Methacrylate Producers Association
Washington, DC

Date

Study Director: Judith C. Stadler — 12/20/93
Judith C. Stadler, Ph.D., D.A.B.T.
Senior Research Toxicologist
Inhalation and Oral Toxicology

Study Sponsor: Neil Krivanek — 12/21/93
Neil Krivanek
Chairman, Scientific Committee
Methacrylate Producers Association

INHALATION SENSORY IRRITATION (RD_{50}) STUDY IN MICE WITH SELECTED METHACRYLATES AND METHACRYLIC ACID

GENERAL INFORMATION

Substance Tested: 2-Propenoic acid, 2-methyl-, methyl ester

Synonyms and Codes:
- o Methyl Methacrylate (MMA)
- o Methyl methacrylate (monomer, inhibited)
- o 2-Methyl-2-propenoic acid, methyl ester
- o MMA

Haskell No.: 19881

CAS Registry No.: 80-62-6

Purity: 100%

Composition:
99.97% Methyl Methacrylate
0.002% Methacrylonitrile
0.003% Isobutanol
0.008% Butanol
0.014% Water
> 7 to 10 ppm MEHQ

Physical Form: Clear Liquid

Substance Tested: 2-Propenoic acid, 2-methyl-, ethyl ester

Synonyms and Codes:
- o Ethyl Methacrylate (EMA)
- o Ethyl methacrylate (monomer)
- o Ethyl 2-methyl-2-propenoate
- o 2-Methyl-2-propenoic acid, ethyl ester
- o E-212

Haskell No.: 19846

CAS Registry No.: 97-63-2

Purity: 99.1%

Composition:
99.13% Ethyl Methacrylate
0.026% Methanol
0.079% Ethanol
0.702% Methyl Methacrylate
0.062% Isopropyl Methacrylate
0.0164% Water
> 7 to 10 ppm MEHQ

Physical Form: Liquid

INHALATION SENSORY IRRITATION (RD_{50}) STUDY IN MICE
WITH SELECTED METHACRYLATES AND METHACRYLIC ACID

GENERAL INFORMATION (continued)

Substance Tested: 2-Propenoic acid, 2-methyl-, butyl ester

Synonyms and Codes:
- o n-Butyl Methacrylate (BMA)
- o n-Butyl methacrylate (monomer)
- o Butyl 2-methyl-2-propenoate
- o N-212

Haskell No.: 19924

CAS Registry No.: 97-88-1

Purity: 99.5%

Composition:
99.49% Butyl Methacrylate
0.327% Methyl Methacrylate
0.130% Butanol
0.005% Isopropyl Methacrylate
0.005% Dibutyl Ether
0.025% Isobutyl Methacrylate
0.008% Unknown
0.0065% Water
> 7 to 10 ppm MEHQ

Physical Form: Liquid

Substance Tested: 2-Propenoic acid, 2-methyl-, dodecyl ester, mixture with tetradecyl 2-methyl-2-propenoate and 2-methyl-2-propenoate ester(s)

Synonyms and Codes:
- o Lauryl methacrylate (LMA)
- o Lauryl methacrylate(,) treated
- o Lauryl methacrylate (or dodecyl 2-methyl-2-propenoate) for 2-propenoic acid, 2-methyl-, dodecyl ester component
- o Tetradecyl methacrylate (or 2-propenoic acid, 2-methyl-, tetradecyl ester) for tetradecyl 2-methyl-2-propenoate component
- o Methacrylate ester(s) for 2-methyl-2-propenoate ester(s) component

Haskell No.: 19763

CAS Registry Nos.: 142-90-5 for lauryl methacrylate component
2549-53-3 for tetradecyl methacrylate component

INHALATION SENSORY IRRITATION (RD_{50}) STUDY IN MICE
WITH SELECTED METHACRYLATES AND METHACRYLIC ACID

GENERAL INFORMATION (continued)

Purity: Not supplied by sponsor

Composition:
64-70% Lauryl methacrylate
24-30% Tetradecyl methacrylate
14% max. Methacrylate ester(s)
< 2% 1-Dodecanol
< 1% 1-Tetradecanol
0.012% max. Hydroquinone

Physical Form: Clear, Yellow Liquid

Substance Tested: 2-Propenoic acid, 2-methyl-

Synonyms and Codes:
- o Methacrylic Acid (MAA)
- o Glacial methacrylic acid
- o Methacrylic acid(, glacial)
- o Methylacrylic acid
- o Lot# 5-031342

Haskell No.: 19762

CAS Registry No.: 79-41-4

Purity: 98.5%

Composition:
98.5% min. Methacrylic acid
0.001% max. Hydroquinone (CAS# 123-31-9)
0.027% max. MEHQ (4-methoxyphenol; CAS# 150-76-5)

Physical Form: Clear Liquid

Stability: All test substances were expected to be stable throughout the exposure phase of the study

Sponsor: Methacrylate Producers Association
1330 Connecticut Avenue N.W., #300
Washington, D.C.

Study Initiated/Completed: 26 August 1992 - 20 December 1993

In-Life Study Initiated/Completed: 6 April 1993 - 4 August 1993

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INHALATION SENSORY IRRITATION (RD_{50}) STUDY IN MICE WITH SELECTED METHACRYLATES AND METHACRYLIC ACID

SUMMARY

Groups of four male Swiss Webster mice were exposed for 30 minutes by inhalation to a concentration of methyl methacrylate (MMA), ethyl methacrylate (EMA), butyl methacrylate (BMA), lauryl methacrylate (LMA), or methacrylic acid (MAA). The MMA, EMA, BMA, and MAA were generated as vapors and concentrations were analyzed by gas chromatography. LMA was generated as an aerosol and the concentrations were determined gravimetrically. Body weights and clinical signs were assessed, and the respiratory frequency of each mouse was determined before, during, and following each exposure. Breathing patterns were also evaluated for signs of sensory or pulmonary irritation. Mice were sacrificed without pathological evaluation immediately after the postexposure monitoring period.

Mice were exposed to 740, 1600, 2900, or 33,000 ppm MMA; 1500, 9100, or 24,000 ppm EMA; and 490, 980, 6300, or 20,000 ppm BMA. The respiratory frequency decreases did not exceed 25% for any of these exposures, and respiratory irritation occurred only briefly in some mice at the initiation of exposure. No RD_{50} values were calculated for these three chemicals.

Exposures to 460, 1500, 2100, 2900, or 3800 mg/m^3 LMA caused both respiratory rate decreases and persistent breathing patterns of sensory irritation at the higher concentrations. The onset of effects occurred slowly and mice were slow to return to normal breathing following exposure. The percent decrease in respiratory rate and the severity of irritation increased with increased concentrations of LMA. The RD_{50} of LMA calculated for this study was 3900 mg/m^3.

Exposures to 4900, 9400, 18,000, 27,000, or 42,000 ppm MAA caused a respiratory rate decrease and breathing patterns of sensory irritation. Onset of effects occurred rapidly at the start of exposures, and dissipated rapidly when the exposures were discontinued. Responses were greater at the higher exposure concentrations. The calculated RD_{50} value was 22,000 ppm.

SUMMARY (Continued)

Of the five materials tested, MMA, EMA, and BMA were considered not to be respiratory irritants. Although sensory irritation was present with LMA and MAA, the high RD_{50} levels indicate that these materials would have a low potential for causing upper respiratory tract irritation.

INHALATION SENSORY IRRITATION (RD_{50}) STUDY IN MICE
WITH SELECTED METHACRYLATES AND METHACRYLIC ACID

SIGNATURE

Date

Authored, Reviewed,
and Approved for Issue
by Study Director: Judith C. Stadler 12/20/93

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INHALATION SENSORY IRRITATION (RD_{50}) STUDY IN MICE
WITH SELECTED METHACRYLATES AND METHACRYLIC ACID

ACKNOWLEDGMENTS

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INHALATION SENSORY IRRITATION (RD_{50}) STUDY IN MICE WITH SELECTED METHACRYLATES AND METHACRYLIC ACID

INTRODUCTION

The purpose of this study was to assess the sensory irritation potential in mice that results from exposure to selected methacrylates and methacrylic acid. The irritation potential of each chemical was determined by measuring the breathing frequency during a series of 30-minute exposures to different concentrations of each test substance and subsequently calculating the concentrations that will produce a 50% depression in respiratory rate (RD_{50}).

MATERIALS AND METHODS

A. Test Substances

The liquid test substances, methyl methacrylate (MMA), ethyl methacrylate (EMA), butyl methacrylate (BMA), lauryl methacrylate (LMA) and methacrylic acid (MAA), were supplied by the Methacrylate Producers Association, Washington, DC. Each test substance was assigned a unique Haskell identification number upon arrival.

B. Animals

Male Swiss Webster mice (Tac:(SW)fBR) were received from Taconic, Germantown, NY. The mice were approximately 28 days old at arrival and weighed between 14 and 24 grams.

Mice for each exposure were selected from a supply of stock animals. Study mice were randomly selected from those that had achieved a body weight of at least 27 grams. Body weight variation was no greater than ± 20% of the mean for each test group.

Swiss Webster mice were selected because they have historically been used in sensory irritation studies and their use is recommended in the American Society

for Testing and Materials Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals (ASTM E981-84).[1]

C. Animal Husbandry

Quarantine. Mice were quarantined after arrival for at least six days prior to testing. They were housed three per cage in 7 1/8 inches x 4 inches x 5 inches suspended, stainless steel, wire-mesh cages and weighed and observed at least twice during the quarantine period. After release from quarantine, the mice were assigned to the general population of stock Swiss Webster mice available for testing.

Housing. Prior to exposure, mice were removed from the animal room to the testing laboratory in shoe-box cages.

Animal Room Environment. The animal room was maintained on a timer-controlled, 12-hour light/12-hour dark cycle. Environmental conditions of the room were targeted within temperatures of 23 ± 2°C and a relative humidity of 50 ± 10%. Excursions outside these ranges were of small magnitude and/or brief duration and did not adversely affect the validity of the study.

Identification. Each mouse was assigned a unique, five-digit identification number which was recorded on tape affixed above the cage. Prior to exposure, mouse tails were coded with water-insoluble markers so that each mouse could be individually identified.

Feed and Water. Purina Certified Rodent Chow® #5002 and tap water from the Wilmington Suburban Water Corporation were available ad libitum, except during exposure.

D. Study Design

Groups of four male Swiss Webster mice were exposed for a single, 30-minute period to a concentration of airborne test substance in air to assess sensory irritation potential. Mice were first weighed then placed into body plethysmographs prior to exposure and acclimated to the exposure chamber for

approximately ten minutes. During this preexposure period, respiratory rates were monitored and recorded to establish a baseline respiration rate. Exposure to the test substance was conducted during the next 30 minutes followed by a postexposure monitoring period of at least 10 minutes. Mice were then removed from the chamber, weighed, and observed for clinical signs of toxicity prior to sacrifice. Respiratory function parameters were monitored during all preexposure, exposure, and postexposure periods.

E. Inhalation Exposure System

1. Atmosphere Generation

A schematic diagram of the generation and exposure systems are in Figure 1. Test atmospheres of MMA, EMA, BMA, and MAA were generated by vaporizing the liquid test substances in a flask heated to 34-76°C (Ace Glass Instatherm®). The test substances were metered into the flask with an infusion pump (Harvard Apparatus Model 22). Filtered air introduced into the flask at approximately 19 L/min carried the vapor through a Teflon® transfer tube into the exposure chamber. Room air was added through the transfer tube to give a total air flow through the exposure chamber of 21 L/min. The chamber concentrations of the four test substances were controlled by varying the feed rates of test substances to the flask (Figure 1).

Test atmospheres of LMA were generated by atomizing the liquid test substance with a nebulizer (Spraying Systems 1/4J; 2850 fluid cap 64 air cap). The test substance was metered into the nebulizer with an infusion pump (Harvard Apparatus Model 22). Approximately 21 L/min of filtered air introduced into the nebulizer, atomized the LMA and carried the aerosol through a glass transfer tube. Room air was added at the glass transfer tube and the resulting aerosol/air mixture passed into the exposure chamber at a rate of 22 L/min. The chamber concentration of LMA was controlled by varying the feed rate of test substance to the nebulizer (Figure 1).

2. Chamber Construction and Design

A cylindrical 2.5-liter exposure chamber, constructed of glass, was used. A glass distribution baffle was built into the chamber about two inches from the air inlet. There were four ports in the exposure chamber to which glass tubes, which served as small body plethysmographs, were connected. Each plethysmograph was approximately three inches long and one inch in diameter. Four mice were placed in the body plethysmographs with heads protruding into the central exposure chamber through individual latex dams (head-only exposure). Sampling ports located on top of the chamber were used to monitor chamber atmospheres.

An exhaust pump pulled air through the exposure chamber. Chamber airflow was monitored and controlled with a calibrated flow meter (Brooks Sho-Rate Model R-6-15-B). Chamber atmosphere was exhausted into the laboratory hood through an emissions abatement train that consisted of a dry-ice cold trap and MSA activated charcoal/particle filter.

3. Respiratory Function Monitoring

Pulmonary function parameters were monitored for all mice. Respiratory frequency was calculated and wave forms were evaluated for pulmonary and sensory irritation.

A pressure transducer was connected to each plethysmograph (Valodyne Model DP-45-14). In turn, each transducer was connected to the pulmonary function monitoring system (Buxco Model LS-20). Physical displacement of the diaphragm in the transducer was converted to an analog signal. The amplified signals were continuously monitored and recorded. Wave forms were output on an eight-channel chart recorder (Graphtec Model WR3310 with black, thermal trace, paper). Charts were saved for later evaluation of wave forms. Signals were analyzed for frequency with system software, which was operated on a personal computer (Dell 486 Model 450/m). The numerical data was saved in computer files for later evaluation.

F. Characterization of Exposure Chamber Atmospheres

1. Vapor Analysis

Test atmospheres of MMA, EMA, BMA, and MAA were determined three times during each exposure. Volumes of chamber atmospheres (4.72 to 11.8 liters) were drawn through a single glass midget impinger that contained acetone as a trapping solvent. The resulting solutions were analyzed by gas chromatography.

Impinger samples were analyzed in duplicate with a gas chromatograph (Hewlett Packard Model 5890) equipped with an integrator (Hewlett Packard Model 3396) and a flame ionization detector. MMA samples were chromatographed isothermally at 75°C on a 30 m x 0.53 mm I.D. polyphenylmethylsiloxane capillary column (Alltech). BMA samples and EMA samples were chromatographed isothermally at 165°C and 80°C, respectively, on a 29 to 30 m x 0.53 mm I.D. polyphenylmethylsiloxane capillary column. MAA samples were chromatographed isothermally at 125°C on a 10 m x 0.53 mm I.D. HP-FFAP capillary column (Hewlett Packard). The atmospheric concentrations of test substances were determined by comparing the detector response of samples with standard curves. Standards were prepared by quantitatively diluting test substances in acetone.

2. Aerosol Analysis

The atmospheric concentration of LMA was determined three to four times during each exposure by gravimetric analysis. Chamber air samples (3.2 to 6.0 liters) were drawn from the chamber through a 25 mm filter cassette which contained a preweighed glass fiber filter (Gelman Type A/E). The filters were weighed on a microbalance (Cahn Model C-31). The atmospheric concentration of LMA was calculated from the difference in the pre- and post-sampling filter weights.

Two samples to determine particle size distribution (mass median aerodynamic diameter and percent particles less than 1, 3, and 10 µm diameter) were taken prior to the first animal exposure with a cyclone preseparator/cascade impactor (Sierra® Series 210) and Constant Flow Air Sampler (Sierra® Series 110).[2]

G. Chamber Environmental Conditions

Temperature was monitored through one of the ports located on the top of the chamber. Temperature was monitored continually with a thermocouple (Omega Type K) and recorded one to four times during each exposure.

Environmental data was monitored from one port located in the exhaust flow. Percent relative humidity was measured during each exposure with a thermo-hygrometer (General Eastern Model 880). Oxygen concentration was measured with an oxygen monitor (Biosystems Model 3100R). Environmental parameters were measured and recorded two to three times during each exposure.

H. Determination of RD_{50}

Respiratory rates in breaths per minute were recorded every 15 seconds during the exposure and postexposure periods. They were compared to baseline respiratory rates recorded during preexposure periods. The baseline respiratory frequency was calculated from a mean of the group for the five minutes directly prior to exposure. For each exposure, a running three-minute mean for the group was calculated for the exposure and postexposure periods. From this data the percent change from baseline respiratory rate for each group was calculated. The lowest three-minute mean was used in the calculation of the RD_{50}. When possible, the RD_{50} value with 95% confidence limits was calculated by using regression analysis that compared the log of the chamber concentration and the percent decrease in respiratory rate.

RECORDS AND SAMPLE RETENTION

All raw data and the final report will be stored in the archives of Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, or in the DuPont Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

RESULTS AND DISCUSSION

A. Exposure Conditions (Tables 1 and 2; Figure 1)

Groups of mice were exposed for 30 minutes each to selected concentrations of the respective test chemicals. Methyl methacrylate (MMA), ethyl methacrylate (EMA), butyl methacrylate (BMA), and methacrylic acid (MAA) were generated as vapors. The highest exposure concentrations for MMA, EMA, and BMA were limited to the highest levels attainable without exceeding the explosive limits. There were four exposures to MMA that ranged in concentration from 740 ppm to 33,000 ppm, three exposures to EMA that ranged from 1500 ppm to 24,000 ppm, four exposures to BMA that ranged from 490 ppm to 20,000 ppm, and six exposures to MAA that ranged from 4900 ppm to 42,000 ppm (Table 1).

Due to the low vapor pressure, lauryl methacrylate (LMA) was generated as an aerosol. The concentrations of the five exposures used to calculate the RD_{50} ranged from 470 mg/m^3 to 3800 mg/m^3. Due to equipment failure, the concentration of LMA fluctuated considerably during one exposure targeted to 500 mg/m^3, and respiratory rate data could not be correlated to exposure concentration. Therefore, these data were not used in the calculations of an RD_{50}, and another exposure targeted to 500 mg/m^3 was conducted. Particle size analysis was performed prior to the first exposures. The mass median aerodynamic diameters and geometric standard deviations of the aerosols, measured on two occasions, were 1.8 $\pm$ 1.7 µm and 1.7 $\pm$ 1.8 µm. At the time particle size samples were taken, the gravimetric concentrations of LMA were approximately 2000 mg/m^3.

The environmental conditions in the exposure chamber were within acceptable comfort ranges for the mice. The animal chamber temperatures ranged from 22 to 29°C, and the percent relative humidity ranged from 24 to 43% (Table 2). Oxygen content for all exposures was 21% and total flow rate through the chamber was maintained between 21 and 22 L/min.

B. Body Weights, Clinical Observations, and Mortality (Table 3)

Three mice died during the study; one during an exposure to MMA and two during exposures to two different concentrations of methacrylic acid. All deaths occurred when mice crawled through the latex neck dam used to maintain an air-tight seal in the plethysmograph. These mice died as a result of chest constriction, and not from effects due to chemical exposure.

Prior to exposure, the mean body weights of the groups of mice ranged from 27 to 30 grams (Table 3). Individually, the majority of mice each lost approximately a gram of body weight during the 30-minute exposures. The group mean percent body weight losses ranged from 1.8 to 5.2%. No severe weight losses due to exposure were evident.

There were no clinical signs observed in mice exposed to any of the concentrations of methacrylates. Mice exposed to concentrations of 18,000 ppm or higher MAA had ocular discharge during and/or following exposures. No other clinical signs of toxicity were observed.

C. Respiratory Irritation (Table 4; Figures 2 through 8; Appendix A)

1. Methyl Methacrylate (MMA)

All groups of mice exposed to MMA had some decrease in respiratory frequency during the exposure when rates were compared to baseline (Figure 2). However, the decreases in respiratory frequency among mice exposed to the two lower concentrations were minimal, and the decrease in frequency was only 18.3% when mice were exposed to 33,000 ppm. Some breathing patterns of mild sensory irritation occurred briefly at the onset of exposures, but did not persist. The limited decrease in respiratory rate at the highest concentration of MMA, and the absence of persistent irritation breathing patterns indicated that MMA is not a respiratory irritant. Extrapolation of the dose-response curve to calculate an RD_{50} value was determined to be inappropriate.

2. Ethyl Methacrylate (EMA)

The maximum decrease in respiratory frequency of mice exposed to 1500 ppm EMA was 4%, and the rates were above baseline during much of the exposure. The respiratory rate decreases were greater at the higher concentrations, but did not exceed 20.7% (Figure 3). There were generally only a few instances of sensory irritation immediately after onset of exposure. No RD_{50} value was calculated, and the material was considered not to be a respiratory irritant at the concentrations tested.

3. Butyl Methacrylate (BMA)

An initial decrease in respiratory rate occurred in all groups of mice exposed to BMA, and the respiratory frequency remained somewhat lower than pre-exposure baseline values throughout the exposure period (Figure 4). The extent of the decrease was similar for all exposure groups, with the maximum decrease ranging from 15.4% to 19.7%. There was no dose-response relationship between the exposure concentration and the percent decrease in respiratory rate. Following exposures, the breathing frequencies increased (Figure 4). In addition, there was no evidence of sensory or pulmonary irritation in the breathing waveforms. Although it is not clear why there was a slight respiratory rate decrease in all exposure groups, it was concluded that BMA does not act as a sensory or pulmonary irritant, and no RD_{50} value was calculated.

4. Lauryl Methacrylate (LMA)

Respiratory frequency gradually declined during each exposure to LMA (Figure 5). The lowest frequencies occurred 25 to 30 minutes into exposure. When exposures were discontinued, frequency increases occurred slowly. Breathing patterns of sensory irritation coincided with the decreases in respiratory frequency. There was a slow onset of abnormal breathing patterns and irritation was most severe near the end of exposure. The RD_{50} of lauryl methacrylate, calculated from five exposures, was 3900 mg/m^3. A dose-response relationship between the LMA concentration and respiratory rate decrease was apparent (Figure 6); however, a 95% confidence interval could not be calculated.

5. Methacrylic Acid (MAA)

During exposures to 4900 ppm MAA, the lowest concentration tested, mice had breathing patterns of mild sensory irritation during the first few minutes. These instances were sporadic. During the exposures to concentrations of 9400 ppm and above, the sensory irritation was moderate to severe, began almost immediately after the onset of exposure (Figure 7), and persisted throughout the 30-minute exposure period. Waveforms returned to normal within a minute after the exposure was discontinued. Respiratory frequency was 8.1% of baseline during the exposure to 4900 ppm. For all other exposures, decreases were 39.6 to 62.8%. Increases in respiratory frequency began to occur after the exposures were terminated. There was a clear dose-response relationship between the MAA concentration and the percent decrease in respiratory frequency (Figure 8). The RD_{50} of methacrylic acid, calculated from six exposures, was 22,000 ppm (95% confidence intervals 15,000 to 40,000 ppm).

CONCLUSION

The three methacrylates, MMA, EMA, and BMA, were not respiratory irritants in mice in this study. Lauryl methacrylate and methacrylic acid were considered sensory irritants; however, the concentrations that produced a 50% decrease in respiratory frequency (RD_{50}) were 3900 mg/m^3 and 22,000 ppm, respectively. Both chemicals produced only mild, brief episodes of sensory irritation at the lowest concentrations tested, which were 460 mg/m^3 for LMA and 4900 ppm for MAA. As a result of this study, lauryl methacrylate and methacrylic acid are considered to have a low potential for causing upper respiratory tract irritation.

REFERENCES

1. Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals. (1984) American Society for Testing and Materials (ASTM).

2. Calculation described in Sierra Instruments, Inc., Bulletin 7-79-219IM, Instruction Manual: Series 210 Ambient Cascade Impactors and Cyclone Preseparators.

SENSORY IRRITATION STUDY WITH METHACRYLATES

TABLES

SENSORY IRRITATION STUDY WITH METHACRYLATES

TABLE 1

CHAMBER CONCENTRATIONS OF TEST SUBSTANCES

TEST SUBSTANCE	CONCENTRATION (ppm) MEAN	S.D.	RD_{50}
MMA	740	82	[a]
	1600	28	
	2900	220	
	33,000	15,000	
EMA	1500	280	[a]
	9100	240	
	24,000	1900	
BMA	490	110	[a]
	980	260	
	6300	310	
	20,000	2000	
LMA	460[b]	260	3900 mg/m^3
	1500	190	
	2100	130	
	2900	290	
	3800	120	
MAA	4900	140	22,000 ppm
	9400	450	
	18,000	650	
	27,000	1400	
	27,000	1500	
	42,000	2900	

[a] An RD_{50} value was not calculated for this test substance.
[b] Values for LMA are expressed as mg/m^3.

SENSORY IRRITATION STUDY WITH METHACRYLATES

TABLE 2

CHAMBER ENVIRONMENTAL CONDITIONS

TEST SUBSTANCE	N[a]	CHAMBER TEMPERATURE (°C)	CHAMBER HUMIDITY (%)	CHAMBER OXYGEN (%)	CHAMBER FLOWRATE[b] (L/min)
MMA	4	23 - 25	27 - 43	21	21
EMA	3	23 - 27	31 - 36	21	21
BMA	4	24 - 27	31 - 40	21	21
LMA	5	22 - 23	24 - 36	21	22
MAA	6	22 - 29	28 - 33	21	21

[a] Number of exposures.
[b] Total flow.

SENSORY IRRITATION STUDY WITH METHACRYLATES

TABLE 3

MEAN BODY WEIGHTS OF MICE

TEST SUBSTANCE	MEAN CONCENTRATION (ppm)	NUMBER	PREEXPOSURE WEIGHT (g)	POSTEXPOSURE WEIGHT (g)	% WEIGHT CHANGE
MMA	740	3	28.3	27.3	-3.5
	1600	4	30.0	28.8	-4.0
	2900	4	29.5	28.5	-3.4
	33,000	4	29.3	28.3	-3.4
EMA	1500	4	28.8	28.0	-2.8
	9100	4	28.0	27.5	-1.8
	24,000	4	28.8	28.0	-2.8
BMA	490	4	27.8	26.8	-3.6
	980	4	28.8	27.8	-3.5
	6300	4	28.3	27.8	-1.8
	20,000	4	28.8	27.3	-5.2
LMA	460[a]	4	27.5	26.5	-3.6
	1500	4	28.3	27.3	-3.5
	2100	4	28.0	26.8	-4.3
	2900	4	27.3	26.8	-1.8
	3800	4	28.0	26.8	-4.3
MAA	4900	4	29.3	28.3	-3.4
	9400	3	28.0	27.0	-3.6
	18,000	4	29.8	28.8	-3.4
	27,000	4	28.5	27.8	-2.5
	27,000	4	28.0	27.3	-2.5
	42,000	3	27.3	26.3	-3.7

[a] Concentration for all LMA exposures expressed as mg/m^3.

SENSORY IRRITATION STUDY WITH METHACRYLATES

TABLE 4

MEAN RESPIRATORY FREQUENCY OF MICE

TEST SUBSTANCE	MEAN CONCENTRATION (ppm)	PRE-EXPSOURE MEAN (bpm)	LOW 3-MIN MEAN (bpm)	PERIOD OF 3-MIN LOW[a] (minutes)	CHANGE[b] (%)
MMA	740	316.1	298.1	22 - 24	5.7
	1600	238.9	216.7	8 - 10	9.3
	2900	299.2	249.9	3 - 5	16.5
	33,000	270.3	220.8	9 - 11	18.3
EMA	1500	295.3	283.4	22 - 24	4.0
	9100	249.8	209.4	3 - 5	16.2
	24,000	289.6	229.7	14 - 16	20.7
BMA	490	323.8	259.9	5 - 7	19.7
	980	299.2	253.1	22 - 24	15.4
	6300	291.1	242.9	5 - 7	16.5
	20,000	283.3	231.0	5 - 7	18.4
LMA	460[c]	313.3	243.9	28 - 30	22.2
	1500	283.7	198.6	28 - 30	30.0
	2100	289.6	202.6	28 - 30	30.0
	2900	290.6	121.6	28 - 30	58.1
	3800	285.1	147.6	25 - 27	48.2
MAA	4900	237.1	218.0	3 - 5	8.1
	9400	323.8	195.6	2 - 4	39.6
	18,000	248.1	137.0	20 - 22	44.8
	27,000	302.8	145.3	22 - 24	52.0
	27,000	245.4	104.0	14 - 16	57.6
	42,000	306.6	114.2	22 - 24	62.8

[a] Minutes into exposure period when lowest 3-minute mean occurred.
[b] Calculated:

$$\frac{\text{Mean Baseline Respiratory Rate - Three Minute Mean Respiratory Rate}}{\text{Mean Baseline Respiratory Rate}} \times 100 =$$

[c] Concentration expressed as mg/m^3.

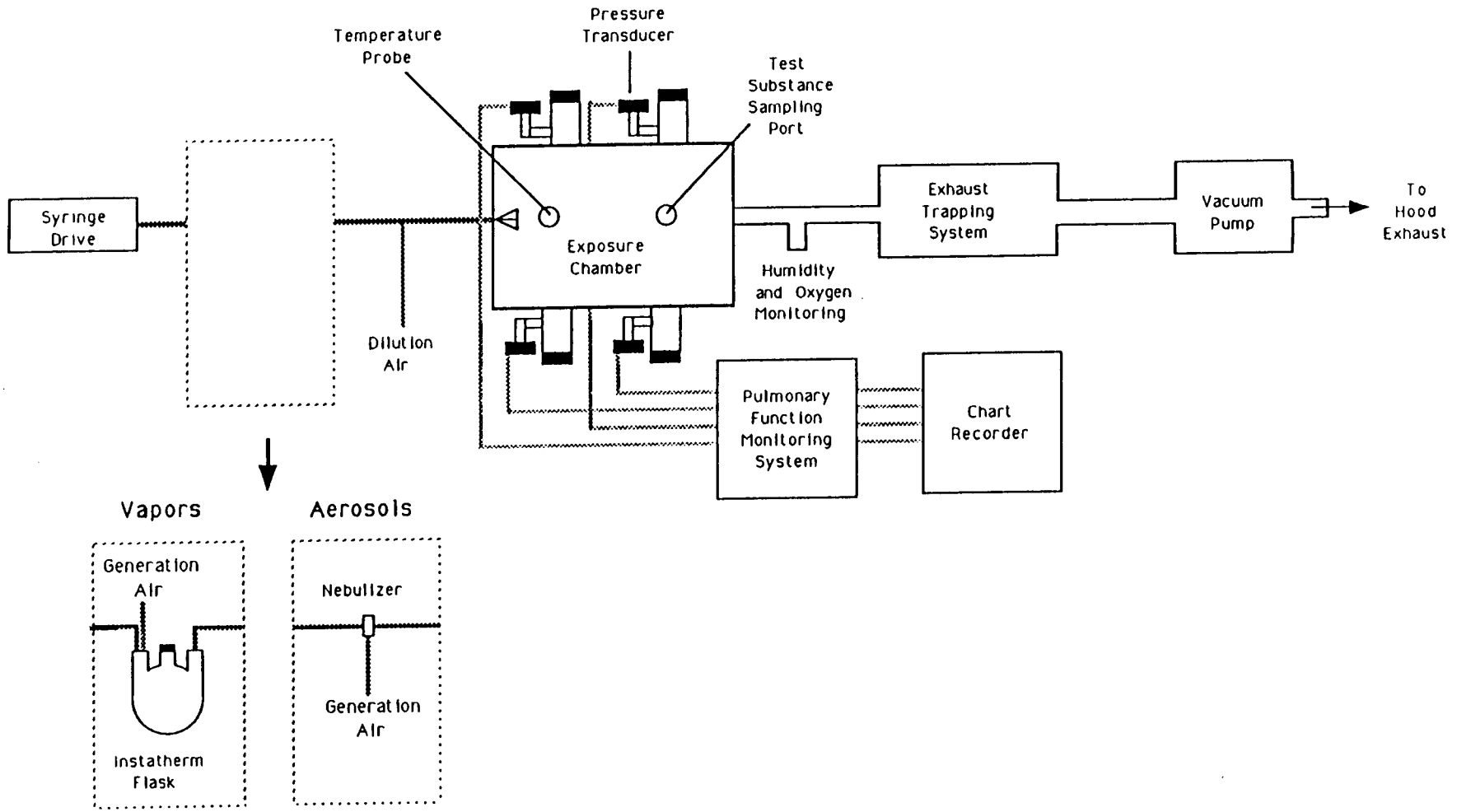
SENSORY IRRITATION STUDY WITH METHACRYLATES

FIGURES

SENSORY IRRITATION STUDY WITH METHACRYLATES

FIGURE 1

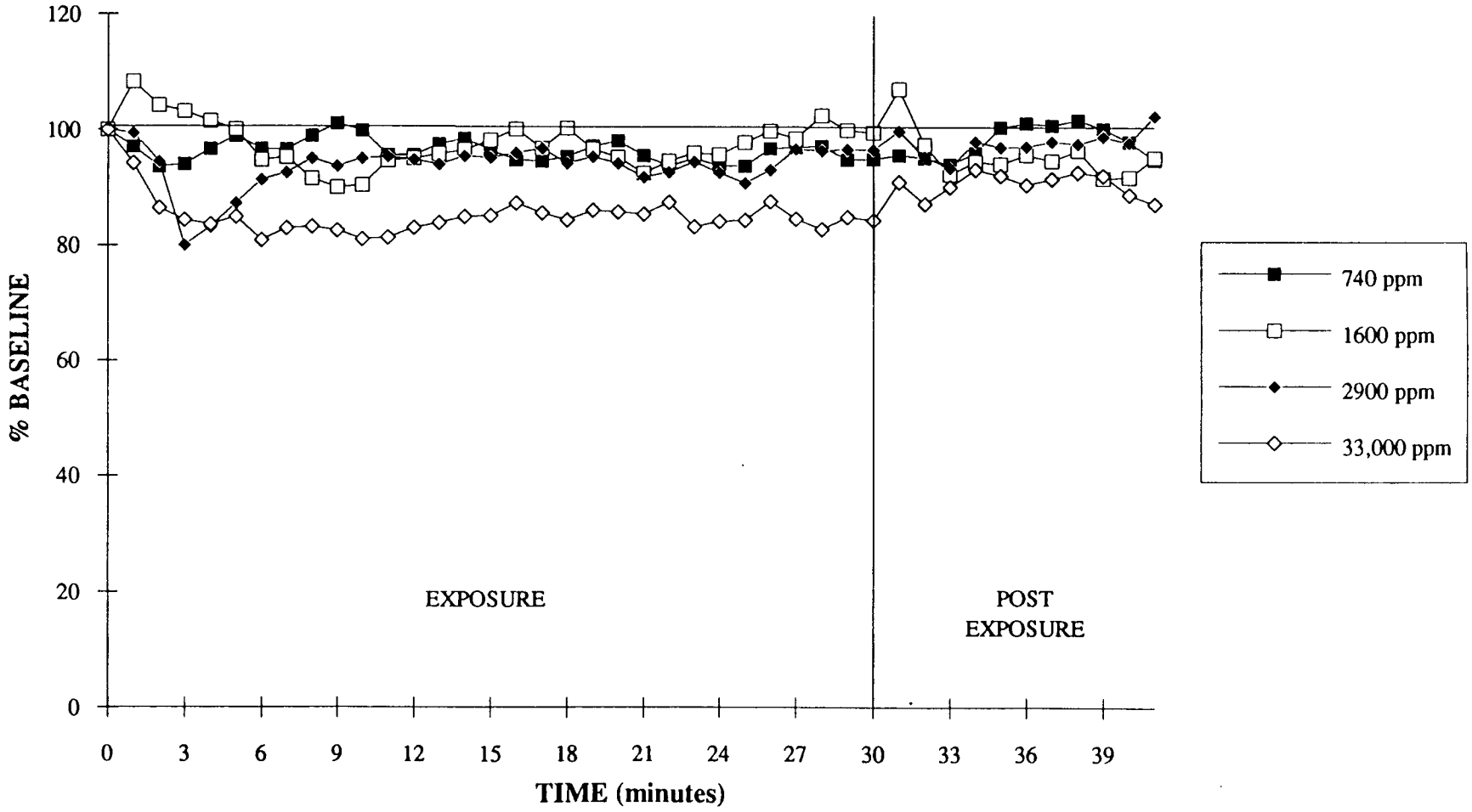
SCHEMATIC OF GENERATION AND EXPOSURE APPARATUS



SENSORY IRRITATION STUDY WITH METHACRYLATES

FIGURE 2

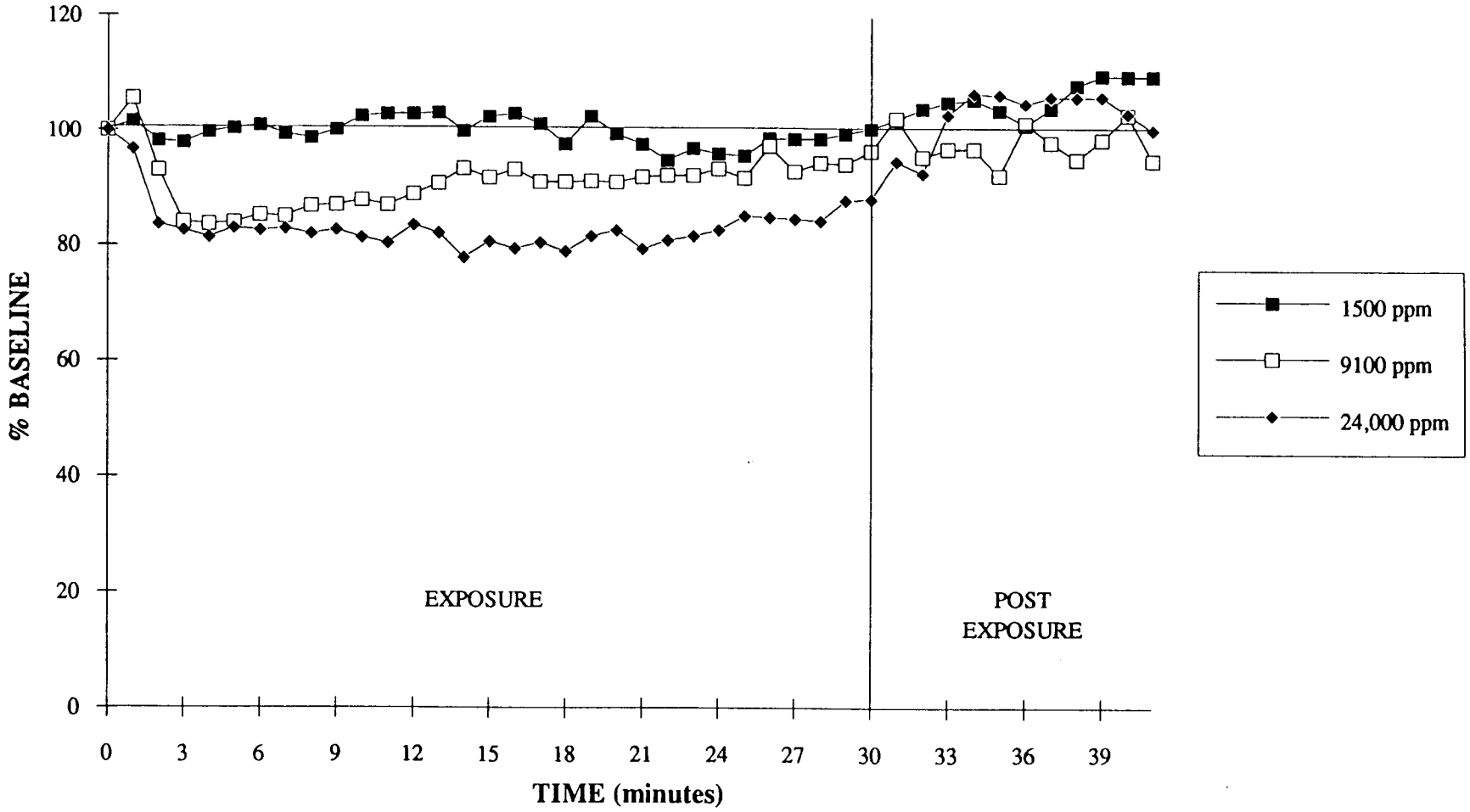
RESPIRATORY FREQUENCY OF MICE EXPOSED TO METHYL METHACRYLATE (MMA)



SENSORY IRRITATION STUDY WITH METHACRYLATES

FIGURE 3

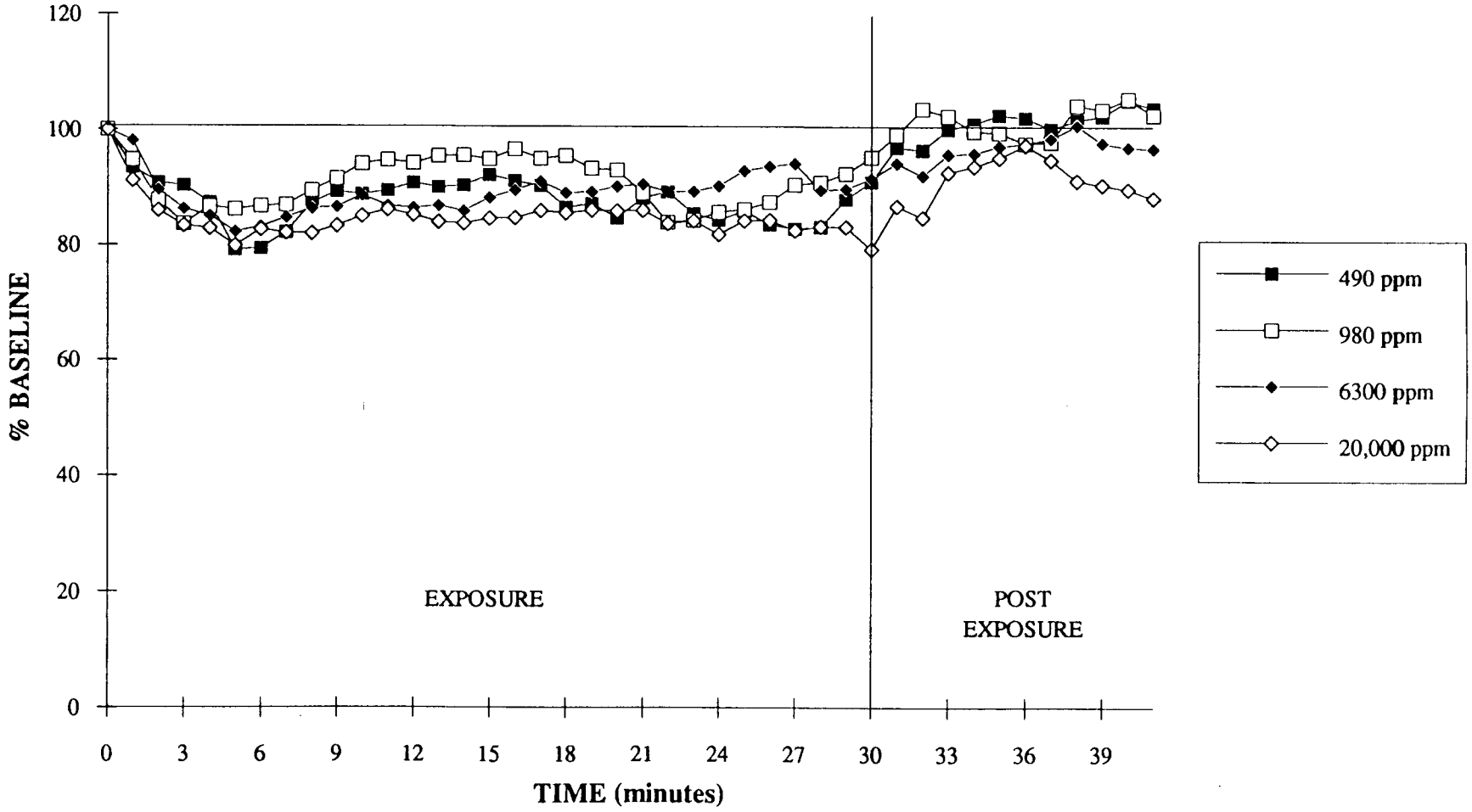
RESPIRATORY FREQUENCY OF MICE EXPOSED TO ETHYL METHACRYLATE (EMA)



SENSORY IRRITATION STUDY WITH METHACRYLATES

FIGURE 4

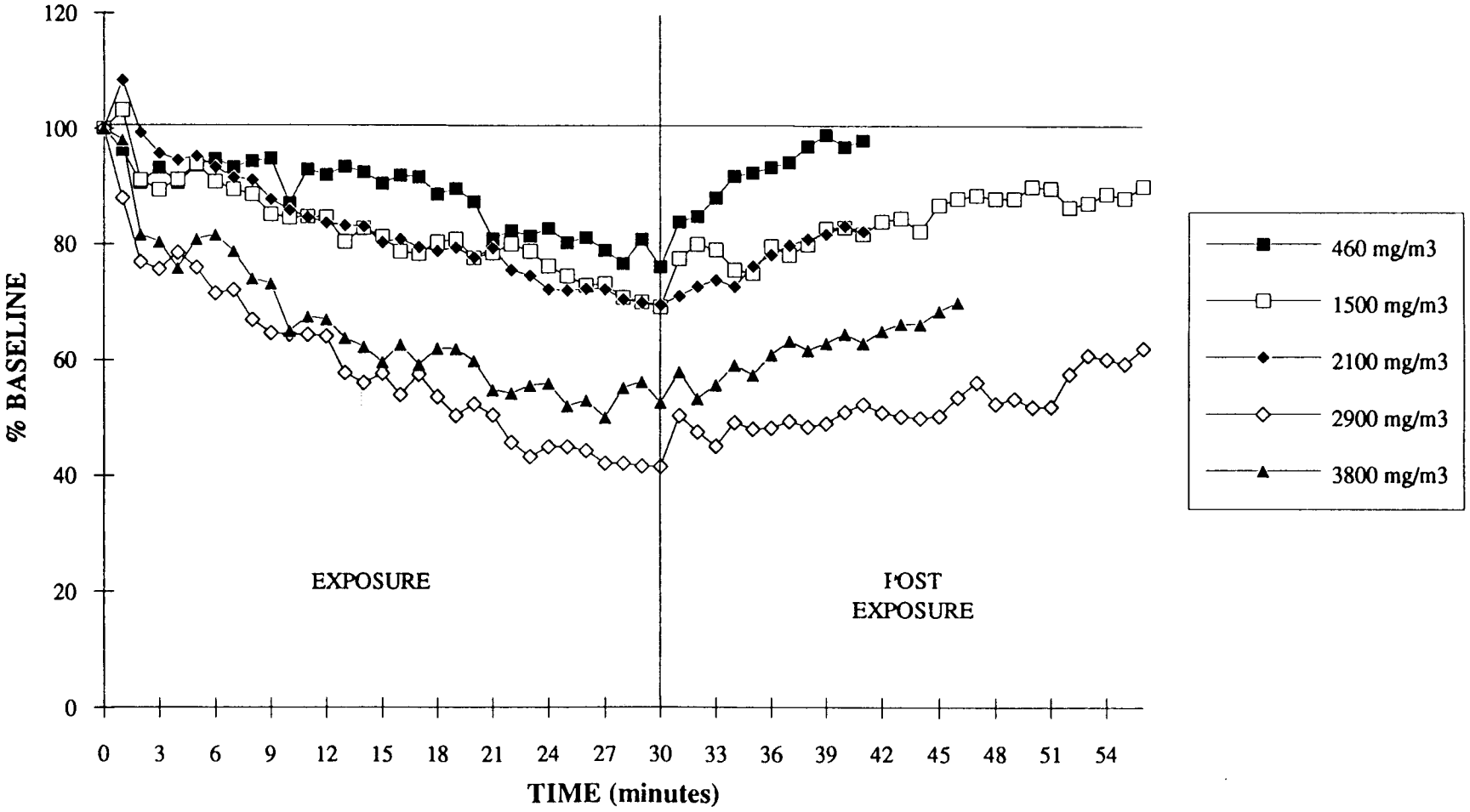
RESPIRATORY FREQUENCY OF MICE EXPOSED TO BUTYL METHACRYLATE (BMA)



SENSORY IRRITATION STUDY WITH METHACRYLATES

FIGURE 5

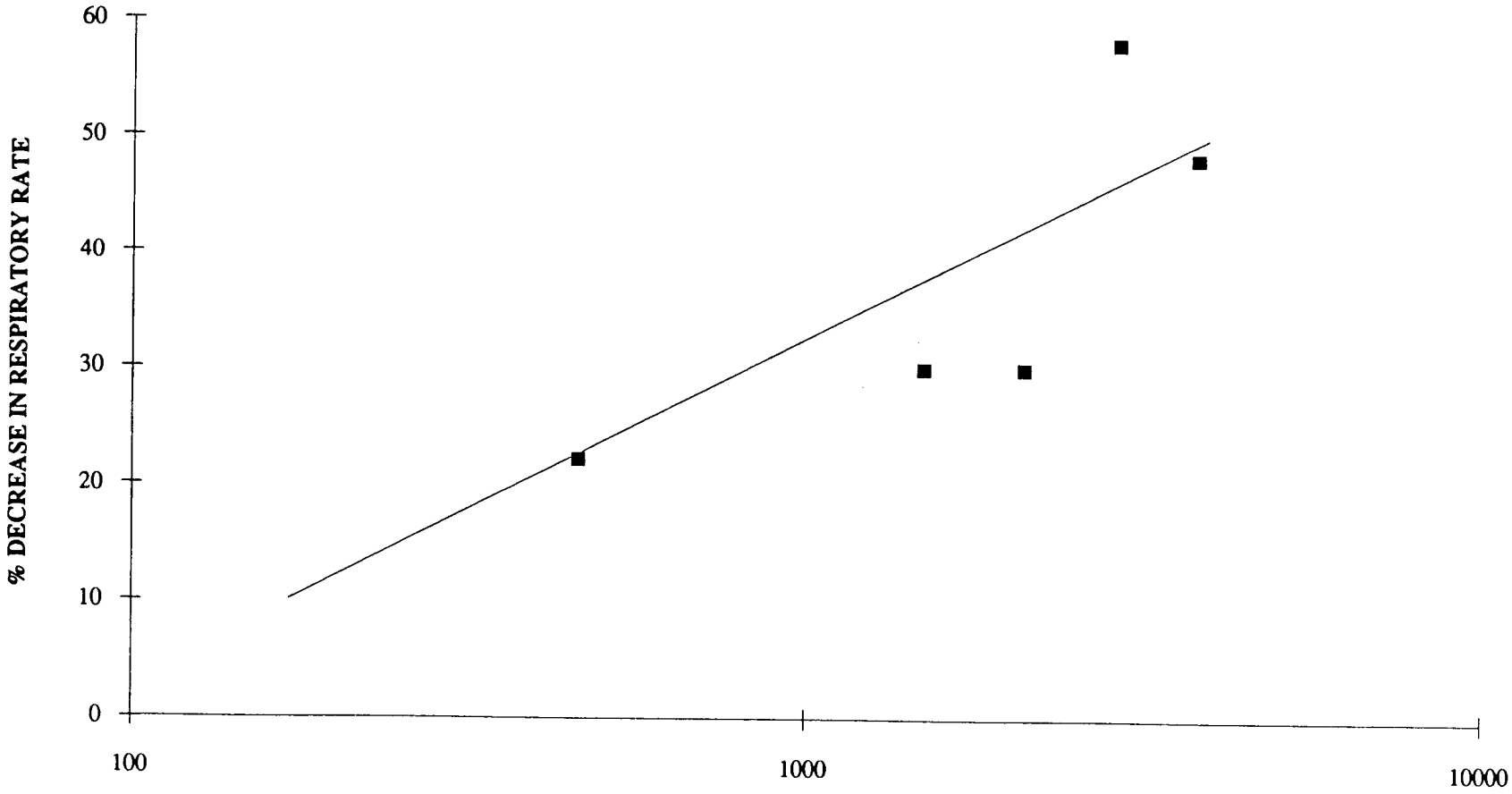
RESPIRATORY FREQUENCY OF MICE EXPOSED TO LAURYL METHACRYLATE (LMA)



SENSORY IRRITATION STUDY WITH METHACRYLATES

FIGURE 6

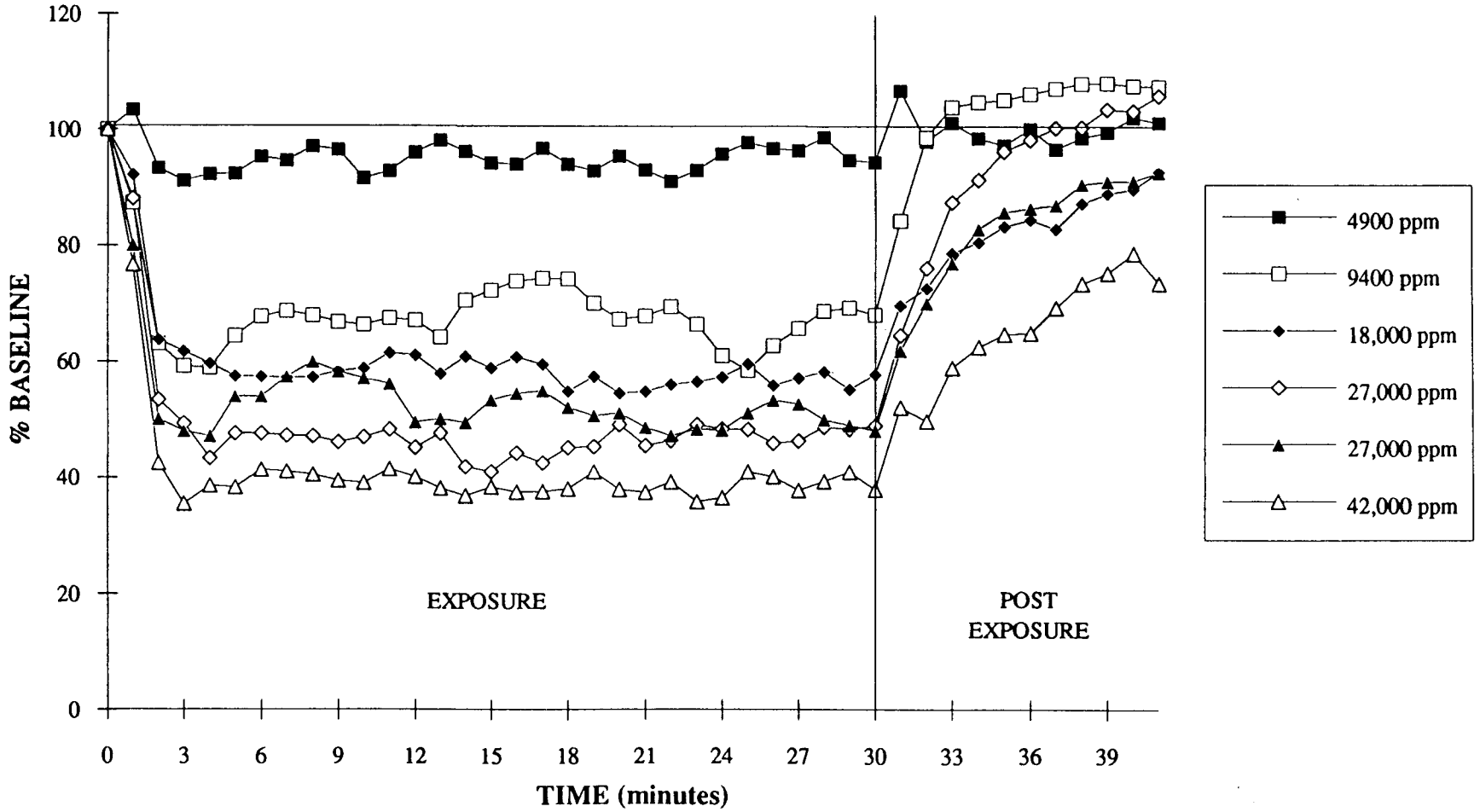
DOSE-RESPONSE CURVE FOR LAURYL METHACRYLATE (LMA)



SENSORY IRRITATION STUDY WITH METHACRYLATES

FIGURE 7

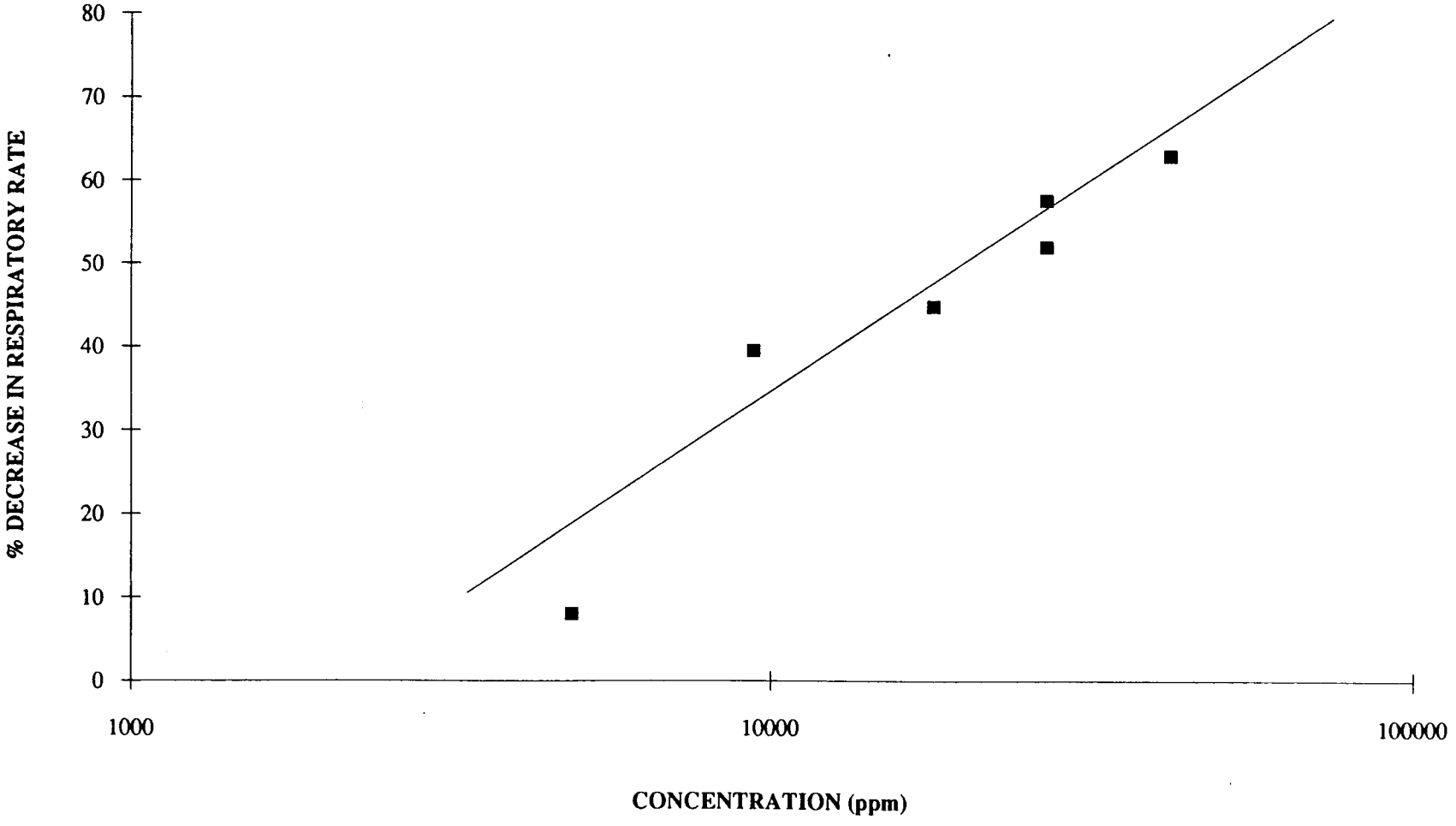
RESPIRATORY FREQUENCY OF MICE EXPOSED TO METHACRYLIC ACID (MAA)



SENSORY IRRITATION STUDY WITH METHACRYLATES

FIGURE 8

DOSE-RESPONSE CURVE FOR METHACRYLIC ACID (MAA)



SENSORY IRRITATION STUDY WITH METHACRYLATES

APPENDIX A

Mean Respiratory Rate Data

SENSORY IRRITATION STUDY WITH METHACRYLATES

MEAN RESPIRATORY RATE DATA

EXPLANATORY NOTES

ABBREVIATIONS

RR - Respiratory Rate

SENSORY IRRITATION STUDY WITH METHACRYLATES

MEAN RESPIRATORY RATE DATA - METHYL METHACRYLATE (MMA)

TIME (MINUTES)	740 ppm RR MEAN	740 ppm % OF BASE	1600 ppm RR MEAN	1600 ppm % OF BASE	2900 ppm RR MEAN	2900 ppm % OF BASE	33,000 ppm RR MEAN	33,000 ppm % OF BASE
BASELINE	316	100.0	239	100.0	299	100.0	270	100.0
1	307	97.1	259	108.4	298	99.5	255	94.3
2	296	93.7	249	104.3	283	94.5	234	86.5
3	297	94.1	247	103.3	240	80.1	228	84.4
4	306	96.7	243	101.5	249	83.2	226	83.6
5	313	99.0	239	100.1	261	87.3	230	85.0
6	306	96.7	227	94.8	273	91.4	219	80.9
7	305	96.6	228	95.3	277	92.6	224	83.0
8	313	99.0	219	91.6	285	95.1	225	83.3
9	320	101.2	215	90.1	281	93.8	223	82.6
10	316	99.9	216	90.5	284	95.1	219	81.1
11	302	95.7	226	94.7	286	95.4	220	81.4
12	303	95.7	227	95.1	284	94.9	225	83.1
13	309	97.6	229	95.9	282	94.1	227	83.9
14	311	98.5	231	96.5	286	95.5	230	85.0
15	305	96.5	235	98.2	285	95.2	230	85.2
16	300	94.8	239	100.1	287	96.1	236	87.3
17	299	94.6	231	96.8	290	96.8	231	85.6
18	302	95.4	240	100.3	282	94.3	228	84.4
19	307	97.2	231	96.7	286	95.4	233	86.1
20	310	98.1	228	95.3	282	94.3	232	85.8
21	302	95.6	221	92.5	275	91.9	231	85.4
22	297	93.8	226	94.6	277	92.7	237	87.5
23	301	95.1	230	96.1	283	94.5	225	83.3
24	297	93.9	229	95.8	277	92.6	228	84.2
25	297	93.8	234	97.9	272	90.8	228	84.4
26	306	96.8	238	99.8	279	93.1	237	87.6
27	307	97.0	235	98.5	289	96.7	229	84.6
28	307	97.2	245	102.5	288	96.4	224	82.8
29	300	94.8	239	99.9	289	96.6	229	84.9
30	300	94.9	238	99.5	289	96.5	228	84.3
31	302	95.6	256	107.0	298	99.7	246	90.9
32	301	95.1	233	97.4	285	95.3	236	87.2
33	297	94.0	220	92.3	279	93.4	244	90.1
34	304	96.0	226	94.4	293	98.0	252	93.1
35	317	100.4	225	94.1	290	97.0	249	92.1
36	320	101.2	229	95.7	291	97.1	245	90.5
37	319	100.8	226	94.6	293	98.0	247	91.5
38	321	101.7	230	96.4	292	97.6	250	92.6
39	317	100.2	219	91.6	296	98.8	249	92.1
40	310	98.0	219	91.8	293	97.8	240	88.8
41	300	94.8	228	95.2	306	102.4	236	87.2

SENSORY IRRITATION STUDY WITH METHACRYLATES

MEAN RESPIRATORY RATE DATA - ETHYL METHACRYLATE (EMA)

TIME (MINUTES)	1500 ppm RR MEAN	1500 ppm % OF BASE	9100 ppm RR MEAN	9100 ppm % OF BASE	24,000 ppm RR MEAN	24,000 ppm % OF BASE
BASELINE	295	100.0	250	100.0	290	100.0
1	300	101.6	264	105.6	280	96.8
2	290	98.2	233	93.1	242	83.6
3	290	98.0	210	84.0	239	82.6
4	294	99.7	209	83.6	236	81.4
5	296	100.4	210	83.9	240	83.0
6	298	101.0	213	85.3	239	82.6
7	294	99.5	213	85.1	240	82.9
8	292	98.8	217	86.9	237	82.0
9	296	100.2	218	87.1	240	82.7
10	303	102.6	220	87.9	236	81.4
11	304	103.0	218	87.1	233	80.4
12	304	103.0	222	89.0	242	83.5
13	305	103.2	227	90.8	238	82.1
14	295	99.9	233	93.4	226	77.9
15	302	102.4	229	91.8	234	80.6
16	304	103.0	233	93.2	230	79.4
17	299	101.2	227	91.0	233	80.4
18	289	97.7	227	91.0	229	78.9
19	303	102.5	228	91.2	236	81.6
20	294	99.5	227	91.0	239	82.6
21	289	97.7	230	92.0	230	79.4
22	280	94.9	231	92.3	234	80.9
23	286	97.0	231	92.3	237	81.7
24	284	96.1	233	93.4	240	82.7
25	283	95.7	229	91.8	247	85.2
26	292	98.7	243	97.4	246	84.9
27	291	98.6	232	92.9	245	84.6
28	291	98.6	236	94.4	244	84.2
29	294	99.5	235	94.2	254	87.8
30	296	100.3	241	96.4	255	88.0
31	301	101.9	255	102.1	274	94.6
32	307	103.8	238	95.4	268	92.5
33	310	105.0	242	96.8	298	102.8
34	311	105.4	242	96.8	309	106.5
35	306	103.5	230	92.2	308	106.3
36	298	101.0	253	101.3	303	104.7
37	307	104.0	245	98.0	307	105.9
38	319	107.9	237	95.0	306	105.8
39	324	109.6	246	98.4	307	105.9
40	323	109.5	257	102.8	298	103.0
41	323	109.5	237	94.8	290	100.1

SENSORY IRRITATION STUDY WITH METHACRYLATES

MEAN RESPIRATORY RATE DATA - BUTYL METHACRYLATE (BMA)

TIME (MINUTES)	490 ppm RR MEAN	490 ppm % OF BASE	980 ppm RR MEAN	980 ppm % OF BASE	6300 ppm RR MEAN	6300 ppm % OF BASE	20,000 ppm RR MEAN	20,000 ppm % OF BASE
BASELINE	324	100.0	299	100.0	291	100.0	283	100.0
1	303	93.5	284	94.8	285	98.1	258	91.2
2	294	90.8	262	87.7	261	89.6	244	86.0
3	293	90.4	250	83.6	251	86.3	236	83.4
4	283	87.3	259	86.7	248	85.1	235	82.9
5	257	79.2	258	86.2	240	82.3	226	79.9
6	257	79.4	260	86.8	242	83.2	234	82.7
7	266	82.2	260	87.0	247	84.8	233	82.1
8	283	87.3	268	89.5	251	86.4	232	82.0
9	289	89.3	274	91.6	252	86.7	236	83.4
10	288	88.9	282	94.2	258	88.7	241	85.1
11	290	89.5	284	94.8	253	86.9	244	86.2
12	294	90.8	282	94.3	252	86.5	242	85.3
13	292	90.1	286	95.5	253	86.9	238	84.0
14	293	90.4	286	95.6	250	85.9	237	83.7
15	299	92.2	284	94.9	257	88.2	240	84.6
16	295	91.1	289	96.6	260	89.5	240	84.7
17	293	90.3	284	95.0	265	91.0	243	85.9
18	280	86.5	286	95.5	259	89.1	242	85.5
19	283	87.2	279	93.3	260	89.2	244	86.0
20	274	84.7	278	93.0	263	90.2	243	85.9
21	286	88.2	266	89.1	264	90.6	244	86.0
22	289	89.2	251	83.9	260	89.2	237	83.7
23	277	85.4	252	84.3	260	89.3	239	84.2
24	273	84.3	256	85.7	263	90.3	232	81.9
25	278	85.9	258	86.2	270	92.9	238	84.2
26	270	83.5	262	87.4	273	93.7	239	84.3
27	268	82.8	271	90.5	274	94.2	234	82.5
28	269	83.1	272	90.8	260	89.5	236	83.2
29	285	87.9	277	92.4	261	89.7	235	83.1
30	294	90.9	285	95.2	266	91.5	224	79.2
31	314	96.9	296	99.1	274	94.2	246	86.7
32	312	96.4	310	103.5	268	92.0	240	84.7
33	324	100.0	306	102.3	278	95.7	262	92.5
34	327	101.0	298	99.6	279	95.9	265	93.6
35	332	102.5	298	99.5	283	97.1	269	95.1
36	330	102.0	292	97.6	284	97.6	276	97.3
37	324	100.0	293	97.9	286	98.4	268	94.7
38	329	101.6	312	104.2	293	100.7	258	91.1
39	331	102.3	309	103.4	284	97.7	256	90.4
40	340	105.1	315	105.3	282	96.9	254	89.6
41	336	103.7	307	102.5	282	96.7	250	88.1

SENSORY IRRITATION STUDY WITH METHACRYLATES

MEAN RESPIRATORY RATE DATA - LAURYL METHACRYLATE (LMA)

TIME (MINUTES)	460 mg/m³ RR MEAN	460 mg/m³ % OF BASE	1500 mg/m³ RR MEAN	1500 mg/m³ % OF BASE	2100 mg/m³ RR MEAN	2100 mg/m³ % OF BASE	2900 mg/m³ RR MEAN	2900 mg/m³ % OF BASE
BASELINE	313	100.0	284	100.0	290	100.0	291	100.0
1	302	96.3	293	103.3	314	108.5	256	88.0
2	284	90.7	258	91.1	288	99.4	223	76.9
3	292	93.3	254	89.4	277	95.8	220	75.7
4	284	90.7	259	91.2	274	94.6	228	78.5
5	294	93.7	267	94.0	276	95.3	221	76.0
6	297	94.8	258	90.8	270	93.4	208	71.5
7	293	93.4	254	89.5	265	91.6	209	72.1
8	296	94.4	252	88.7	264	91.2	195	67.0
9	297	94.9	242	85.2	254	87.8	188	64.7
10	273	87.2	240	84.6	249	85.9	187	64.4
11	291	93.0	241	84.8	245	84.7	187	64.4
12	288	92.1	240	84.7	242	83.7	186	64.1
13	293	93.5	228	80.4	241	83.3	168	57.8
14	290	92.5	235	82.8	241	83.1	163	56.1
15	284	90.6	231	81.3	232	80.3	168	57.7
16	288	92.0	223	78.7	234	80.9	157	54.0
17	287	91.7	222	78.3	230	79.5	167	57.5
18	278	88.7	228	80.3	228	78.8	156	53.6
19	281	89.6	229	80.8	230	79.4	147	50.4
20	274	87.3	220	77.6	225	77.7	152	52.4
21	253	80.9	223	78.4	230	79.3	147	50.5
22	258	82.3	227	80.0	219	75.5	133	45.8
23	255	81.4	223	78.7	216	74.6	126	43.3
24	259	82.7	216	76.2	209	72.2	131	45.0
25	251	80.2	211	74.5	209	72.0	131	45.0
26	254	81.1	207	72.9	209	72.3	129	44.3
27	247	78.9	207	73.1	209	72.2	122	42.2
28	240	76.6	201	70.8	204	70.5	123	42.2
29	253	80.8	199	70.0	202	69.9	121	41.7
30	238	76.1	196	69.2	201	69.5	121	41.6
31	263	83.8	220	77.5	206	71.1	146	50.4
32	266	84.8	227	80.0	211	72.7	138	47.6
33	276	88.0	224	79.0	214	73.8	131	45.1
34	288	91.8	214	75.5	211	72.7	143	49.2
35	290	92.4	212	74.9	221	76.2	139	48.0
36	292	93.3	226	79.6	227	78.2	140	48.2
37	295	94.2	222	78.1	231	79.8	144	49.4
38	304	96.9	227	79.9	234	80.8	141	48.4
39	310	98.9	234	82.6	237	81.7	142	49.0
40	303	96.8	235	82.8	241	83.1	148	51.0
41	307	98.0	232	81.7	238	82.1	152	52.3

SENSORY IRRITATION STUDY WITH METHACRYLATES

MEAN RESPIRATORY RATE DATA - LAURYL METHACRYLATE (LMA)

TIME (MINUTES)	460 mg/m³ RR MEAN	460 mg/m³ % OF BASE	1500 mg/m³ RR MEAN	1500 mg/m³ % OF BASE	2100 mg/m³ RR MEAN	2100 mg/m³ % OF BASE	2900 mg/m³ RR MEAN	2900 mg/m³ % OF BASE
42			238	83.8			148	50.9
43			239	84.4			146	50.2
44			233	82.1			145	49.9
45			246	86.7			146	50.3
46			249	87.8			156	53.5
47			251	88.4			163	56.1
48			249	87.8			152	52.4
49			249	87.8			155	53.2
50			255	89.9			151	51.8
51			254	89.6			151	51.9
52			245	86.3			167	57.5
53			247	87.1			176	60.7
54			252	88.7			175	60.1
55			249	87.9			172	59.3
56			255	90.0			180	62.0

SENSORY IRRITATION STUDY WITH METHACRYLATES

MEAN RESPIRATORY RATE DATA - LAURYL METHACRYLATE (LMA)

TIME (MINUTES)	3800 mg/m³ RR MEAN	3800 mg/m³ % OF BASE	TIME (MINUTES)	3800 mg/m³ RR MEAN	3800 mg/m³ % OF BASE
BASELINE	285	100.0			
1	280	98.1	42	185	65.0
2	233	81.7	43	189	66.2
3	229	80.4	44	188	66.1
4	216	75.9	45	195	68.4
5	231	80.9	46	199	69.9
6	233	81.7			
7	225	78.8			
8	211	74.1			
9	209	73.2			
10	186	65.1			
11	193	67.5			
12	191	67.1			
13	182	63.9			
14	178	62.3			
15	170	59.7			
16	179	62.7			
17	169	59.2			
18	177	62.1			
19	177	62.0			
20	171	59.9			
21	157	54.9			
22	155	54.3			
23	159	55.6			
24	160	56.0			
25	149	52.1			
26	152	53.1			
27	143	50.1			
28	158	55.2			
29	160	56.3			
30	150	52.7			
31	166	58.0			
32	152	53.3			
33	159	55.7			
34	169	59.1			
35	164	57.4			
36	174	60.9			
37	180	63.3			
38	176	61.7			
39	179	62.9			
40	184	64.5			
41	179	62.9			

SENSORY IRRITATION STUDY WITH METHACRYLATES

MEAN RESPIRATORY RATE DATA - METHACRYLIC ACID (MAA)

TIME (MINUTES)	4900 ppm RR MEAN	4900 ppm % OF BASE	9400 ppm RR MEAN	9400 ppm % OF BASE	18,000 ppm RR MEAN	18,000 ppm % OF BASE	27,000 ppm RR MEAN	27,000 ppm % OF BASE
BASELINE	237	100.0	324	100.0	248	100.0	245	100.0
1	245	103.4	283	87.3	229	92.2	216	88.1
2	221	93.3	204	63.1	158	63.8	131	53.4
3	216	91.1	192	59.2	153	61.8	121	49.4
4	219	92.3	191	58.9	148	59.7	106	43.4
5	219	92.4	209	64.4	143	57.5	117	47.7
6	226	95.3	219	67.7	142	57.4	117	47.7
7	224	94.6	222	68.7	142	57.2	116	47.3
8	230	97.1	220	67.9	142	57.3	116	47.2
9	229	96.5	216	66.8	145	58.4	113	46.1
10	217	91.6	215	66.3	146	58.8	115	47.0
11	220	92.8	218	67.4	153	61.5	119	48.3
12	228	96.1	217	67.1	152	61.1	111	45.2
13	233	98.1	208	64.1	144	57.9	117	47.7
14	228	96.2	228	70.5	151	60.8	103	41.9
15	223	94.2	234	72.2	146	58.8	101	41.0
16	223	94.0	239	73.8	151	60.7	108	44.2
17	229	96.7	241	74.3	148	59.5	104	42.5
18	223	94.0	240	74.2	136	54.9	111	45.2
19	220	92.8	227	70.0	142	57.4	111	45.4
20	226	95.4	218	67.3	136	54.6	121	49.2
21	221	93.0	220	67.8	136	54.9	112	45.6
22	216	91.0	225	69.4	139	56.1	114	46.4
23	220	92.9	215	66.4	140	56.6	121	49.2
24	227	95.8	198	61.0	142	57.3	119	48.4
25	232	97.8	189	58.5	148	59.6	119	48.3
26	229	96.7	203	62.7	139	55.9	113	46.0
27	228	96.3	213	65.7	142	57.1	114	46.3
28	234	98.6	222	68.6	144	58.2	120	48.7
29	224	94.6	224	69.2	137	55.1	119	48.3
30	224	94.3	220	67.9	143	57.7	120	48.9
31	253	106.7	272	84.1	172	69.5	158	64.4
32	232	97.9	319	98.5	180	72.5	187	76.0
33	240	101.1	336	103.8	195	78.5	214	87.3
34	233	98.4	339	104.7	199	80.4	224	91.2
35	230	97.2	340	105.1	206	83.2	236	96.2
36	237	100.0	343	106.1	209	84.3	241	98.1
37	229	96.5	346	107.0	205	82.7	246	100.0
38	234	98.5	349	107.9	216	87.2	246	100.3
39	236	99.5	350	108.0	221	88.9	254	103.4
40	242	102.0	348	107.5	222	89.6	253	103.2
41	240	101.0	348	107.4	230	92.6	260	105.8

SENSORY IRRITATION STUDY WITH METHACRYLATES

MEAN RESPIRATORY RATE DATA - METHACRYLIC ACID (MAA)

TIME (MINUTES)	27,000 ppm RR MEAN	27,000 ppm % OF BASE	42,000 ppm RR MEAN	42,000 ppm % OF BASE
BASELINE	303	100.0	307	100.0
1	243	80.1	235	76.7
2	152	50.1	130	42.5
3	145	48.0	109	35.5
4	143	47.2	119	38.7
5	163	54.0	118	38.4
6	163	54.0	127	41.4
7	174	57.4	126	41.1
8	182	60.0	124	40.6
9	176	58.3	121	39.5
10	173	57.1	120	39.1
11	170	56.2	127	41.5
12	150	49.6	123	40.2
13	152	50.1	117	38.2
14	150	49.5	113	36.9
15	161	53.3	118	38.4
16	165	54.5	115	37.5
17	167	55.0	115	37.6
18	158	52.1	117	38.1
19	153	50.7	126	41.0
20	155	51.2	117	38.0
21	147	48.7	115	37.5
22	143	47.3	120	39.3
23	147	48.4	110	35.9
24	146	48.2	112	36.6
25	155	51.2	126	41.0
26	161	53.3	123	40.2
27	160	52.7	116	37.8
28	151	49.9	120	39.3
29	148	49.0	125	40.9
30	145	48.0	116	37.9
31	187	61.8	159	52.0
32	212	69.9	153	49.7
33	233	76.8	180	58.8
34	250	82.7	191	62.4
35	259	85.6	198	64.6
36	261	86.3	199	64.8
37	263	86.9	212	69.2
38	274	90.5	225	73.3
39	275	90.9	230	75.1
40	275	91.0	241	78.5
41	280	92.5	225	73.4